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Biological Remediation of Contaminated Sediments, with Special Emphasis on the Great Lakes: Report of a Workshop, Manitowoc, Wisconsin, July 17-19, 1990

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16. ABSTRACT

These proceedings describe a workshop at which biological remediation of contaminated sediments was discussed. For the purpose of the workshop, contaminated sediments of primary interest were those within six of the Areas of Concern (AOC) identified in the U.S./Canada International Joint Commission's Great Lakes Water Quality Board; five of these AOC are priority concerns of the U.S. Environmental Protection Agency's Assessment and Remediation of Contaminated Sediments (ARCS) Program.

The workshop was organized around four topic areas: (1) Overview of the Areas of Concern, (2) Biological degradation of PCBs; (3) Biological degradation of PAHs, and (4) Biological treatment of metal species. For the first topic area, presentations were made describing site characteristics of the Ashtabula River, OH; the Buffalo River, NY; the Sheboygan River, WI; the Grand Calumet River, IN; the Saginaw River and Bay, MI; and the Hamilton Harbor, Ontario, Canada. For the remaining topic areas, presentations were made by investigators actively involved in either bench, pilot, or full-scale studies concerning these areas. This document provides extended abstracts and brief summaries of the presentations and discussion sessions at the workshop.

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Biological Remediation of Contaminated Sediments, with Special Emphasis on the Great Lakes

Report of a Workshop

Manitowoc, Wisconsin
July 17-19, 1990

Edited by C.T. Jafvert and J.E. Rogers

Co-Chairmen:
Chad T. Jafvert and John E. Rogers
Environmental Research Laboratory
U.S. Environmental Protection Agency
Athens, Georgia 30613

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FOREWORD

As environmental controls become more costly to implement and the penalties of judgement errors become more severe, environmental quality management requires more efficient analytical tools based on greater knowledge of the environmental phenomena to be managed. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, research is performed on the biological remediation of contaminated sediments.

The Assessment and Remediation of Contaminated Sediments (ARCS) Program is a major activity of the U.S. Environmental Protection Agency that evaluates and demonstrates remediation alternatives for contaminated sediments within the Great Lakes Basin and associated risk assessments. In the summer of 1990, more than 60 scientists from the United States, Canada, and The Netherlands participated in a special workshop to present the current state-of-the-science concerning the biodegradation of polychlorinated biphenyls and polyaromatic hydrocarbons and the biological treatment of metal species. This proceedings provides a synopsis of the information exchanged at that workshop.

Rosemarie C. Russo, Ph.D.
Director
Environmental Research Laboratory
Athens, Georgia

ABSTRACT

These proceedings describe a workshop held July 17-19, 1990 in Manitowoc, WI, at which biological remediation of contaminated sediments was discussed. For the purpose of the workshop, contaminated sediments of primary interest were those within six of the Areas of Concern (AOC) identified by the U.S./Canada International Joint Commission's Great Lakes Water Quality Board; five of which are priority concerns of the U.S. Environmental Protection Agency's Assessment and Remediation of Contaminated Sediments (ARCS) program.

The workshop was organized around four topic areas: (1) Overview of the Areas of Concern; (2) Biological degradation of PCBs; (3) Biological degradation of PAHs; and (4) Biological treatment of metal species. For the first topic area, presentations were made describing site characteristic of the Ashtabula River, OH; Buffalo River, NY; Sheboygan River, WI; Grand Calumet River, IN; Saginaw River and Bay, MI; and Hamilton Harbor, Ontario, Canada. For the remaining topic areas, presentations were made by investigators actively involved in either bench, pilot, or full-scale studies concerning these areas. In this document extended abstracts written by the presenters are given, as well as brief summaries of the presentations and discussion sessions.

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We gratefully acknowledge the efforts of all those individuals who contributed in one form or another to the origination of this report. The Workshop and this report, truly, were group projects. Recognition is extended to David Cowgill and Paul Horvatin of E.P.A.'s Great Lakes National Program Office (GLNPO) and members of GLNPO's Engineering and Technology Workgroup, and its Chairman, Steve Yaksich of the U.S. Army Corp of Engineers, Buffalo District, for their support and planning input. Also, we deeply appreciate the support and planning input provided by Griff Sherbin and Ian Orchard of Environment Canada. Direction by all these individuals has enhanced this report considerably by their endeavor to assure its applicability to contaminated sediment scenarios within the Great Lakes. Appreciation is given to Paulette Altringer of the Bureau of Mines, Salt Lake City Research Center, who was instrumental in organizing the Metals session. Janice Heath of Technology Applications Inc. and Patricia Van Hoof of The University of Georgia provided indispensable assistance in making Workshop arrangements and coordinating activities during the Workshop. In particular, we wish to acknowledge Janice Heath for her singular effort of synthesizing the many diverse forms of material submitted by the speakers into a consistently formatted and understandable document.

1 INTRODUCTION

The current state-of-the-science of biological remediation of contaminated sediments was discussed in a workshop held July 17 - 19, 1990, in Manitowoc, WI. Special emphasis was devoted to remediation alternatives for sediments within the Great Lakes Basin. The workshop was supported by the U.S. EPA's Great Lakes National Program Office, through the Assessment and Remediation of Contaminated Sediments (ARCS) Program, by Environment Canada, and by EPA's Biosystems Technology Development Program. More than 60 scientists from state and federal agencies, academia, and the private sector from the United States, Canada, and The Netherlands participated.

For the purpose of the workshop, the sediments of primary interest were those within the Areas of Concern identified by the U.S./Canada International Joint Committee's Great Lakes Water Quality Board. Most of the 42 Areas of Concern are located in harbors, bays, or river mouths; 25 are located within U.S. waters, 12 within Canadian waters, and 5 within international channels. Remedial Action Plans currently are being developed for these areas under the 1987 revision of the Great Lakes Water Quality Agreement. A major purpose of EPA's ARCS Program is to evaluate remediation alternatives for the cleanup of these sites with special emphasis given to five sites. These five are Ashtabula River, OH; Buffalo River, NY; Sheboygan River, WI; Grand Calumet River, IN; and Saginaw River and Bay, MI. Two of these five overlap EPA Superfund sites to some extent.

The Workshop was organized around four topic areas:

I. Overview of the Primary Areas of Concern
II. Biological Degradation of PCBs, Laboratory and Field Studies
III. Biological Degradation of PAHs, Laboratory and Field Studies
IV. Biological Treatment of Metal Species

For the first topic area, presentations were made describing site characteristics of the five primary U.S. Areas of Concern and for Hamilton Harbour, Ontario. Major contaminants within these and other areas include polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and various heavy metal species. The toxicity and recalcitrant nature of these compounds have caused serious environmental concern. Moreover, these classes of contaminants present serious and rather complex treatability problems for essentially all remediation technologies (including biological processes).

For the remaining topic areas, presentations were made by investigators actively involved in either bench-, pilot-, or full-scale studies within these topic areas. To focus dialogue on the Workshop intent, the participants were asked to address or keep in mind the following general questions during presentations and discussion periods:

1. What stage of development have specific bioremediation technologies reached (e.g., laboratory research, laboratory-field development, or full-scale operation)?

2. Which development directions are logical continuations for the specific laboratory studies (e.g., above ground reactor treatment, *in situ* treatment, CDF modification, land farming, or other)?

3. What level of development is necessary before a full-scale application of this technology is feasible?

4. What are the rate limiting factors controlling the optimization of the laboratory or field

process? These factors may be site characteristic considerations, process operation considerations, or both.

5. What types of costs are or will be associated with the development of proposed treatment (e.g., capital, labor, maintenance)? How is this cost dependent on site location and characteristics?

6. What other waste streams may be generated? What losses to the environment will result from specific treatment alternatives? What contaminant residues will result?

7. What concerns you the most regarding the application of specific bioremediation technologies to the problems associated with Great Lakes sediments?

8. Given the dissimilarity between bioremediation technologies and other physical or chemical treatment technologies, how should one compare the environmental and financial costs associated with each?

These questions were intended to be used as a guideline. Answers to some were addressed in detail for specific bioremediation alternatives and are addressed in the Summary sections and in several of the Abstracts. The answers to others were only alluded to or are presently unknown. To a large extent this is because biological remediation to treat contaminated sediments may take several forms. Each form (or process design) has its own list of factors or parameters associated with it that must be considered when optimizing treatment. Hence, there are generally no simple answers to questions regarding the feasibility of biological remediation alternatives. Sediments are generally not contaminated with single compounds or even classes of compounds. Additionally, the interactions among the various organisms responsible for the decomposition of anthropogenic compounds and the sediment matrix are unknown in many cases. Such intricacies make a concise summary of this diverse workshop difficult; however, several general conclusions can be drawn. We hope this Proceedings will benefit scientists and engineers who must make choices among diverse treatment technologies. A brief summary of the Proceedings of this workshop has been published by C. T. Jafvert (*J. Great Lakes Res.* 16(3):337-338, 1990).

Chad T. Jafvert
John E. Rogers

September 1990

2 SUMMARY

2.1 Areas of Concern

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The locations of the 42 Areas of Concern (AOC) identified by the U.S./Canada International Joint Commission's Great Lakes Water Quality Board are illustrated in Figure 2.1.1. Environmental characteristics of the five U.S. AOC whose names are given in this figure were described by either the State AOC Remedial Action Plan Coordinator or the Superfund Site Coordinator for the adjacent Superfund site. A description of Hamilton Harbour, Ontario, was given by Thomas Murphy of Environment Canada.

John McMahon, of the New York State Department of Environmental Conservation (DEC), presented information on the Buffalo River AOC and the Remedial Action Plan Strategy. The Buffalo River, located in western New York State, flows into Lake Erie near the mouth of the Niagara River. Historically, the Buffalo River was used by industries as a transportation channel, a source of cooling water, and a means of disposing of wastewater. These industries were involved in chemical manufacturing (dyes and acids), coke and steel production, and oil refining. Only two of these facilities are still in operation and they are under strict pollution control regulations. Over the years, however, the pollution these industries generated contaminated the river sediments and left hazardous waste on the banks. The bottom sediments contain PAHs, PCBs, and heavy metals, which continue to be a source of contamination to the Buffalo River, as are hazardous waste sites along its banks. Another source of pollution to the river are combined sewer overflows that release dilute sewage and associated contaminants into the river during storm events. In order to restore the Buffalo River's integrity, a Remedial Action Plan (RAP) strategy was devised. The short term goal is to restore the river's ecological system, while the long term goal is to eliminate the sources of pollutants to the river. Presently, the DEC has committed to several initial actions recommended by the RAP for dealing with the sources of contaminants and remediation of the area.

An overview of the Fields Brook Superfund site and the Ashtabula River AOC was given by Pete Sanders of the U. S. Environmental Protection Agency, Region V. The area involved is located in northeast Ohio. Fields Brook flows into the Ashtabula River about 8000 feet from the point at which the river empties into Lake Erie. The Fields Brook site has been on the National Priorities list since the first list was established under Superfund in 1983. Contamination of sediments in this area has resulted from a variety of chemical manufacturers located along Fields Brook. The sediment contaminants include a variety of organic compounds and heavy metals. Clean up and remediation efforts for the Superfund site will involve excavating, dewatering, and either landfilling or thermally treating the contaminated sediment. The option to landfill or thermally treat the sediment will be decided after investigating the mobility of the contaminants, the toxicity and concentration of the contaminants, and the concentration of PCBs. Thermal treatment was indicated in the Record of Decision (ROD) signed by the U.S. EPA in 1986. The ROD also advised a Remedial Investigation (RI)/Feasibility Study (FS) to recognize current sources of contamination to Fields Brook and to examine the extent of contamination to the Ashtabula River. The Ashtabula River investigation included sediment, water, and fish sampling, and started late in 1989. A plan is being developed by the Army Corps of Engineers to dredge the upper portion of the contaminated sediment from the river and place it in a confined disposal area.

Robert Bunner of the Indiana Department of Environmental Management gave an overview of the Indiana Harbor/Grand Calumet River AOC. He showed the initial segment of a video tape entitled "The Grand Calumet River, A River of Contradictions." A copy of this tape can be obtained by contacting Robert Bunner, Indiana Department of Environmental Management, 105 S. Meridian Street, Indianapolis, Indiana 46225. The geographical region associated with the harbor and river has had a long history of industrial activity, beginning in the early part of this century. In fact, over the decades, this portion of the Grand Calumet River has been modified dramatically from its preindustrial state. The major industrial complexes associated with this site over this time period are steel plants. Currently, the dredging of contaminated sediments is proposed in the harbor primarily for navigational purposes, and in the river for remediation purposes. Within this system, deposition of sediments is to the point where it is no longer safe to navigate large ships. The sediments within the river and harbor are contaminated with PCBs, PAHs, and heavy metals including cadmium, chromium, and lead. To give some historical context as to the industrial nature of this area, it is estimated that the land extending one mile from Lake Michigan in the harbor area consists of fill generated from the steel industries over the decades.

Bonnie Eleder of the U.S. Environmental Protection Agency, Region V, presented an overview of the Sheboygan River and Harbor Area of Concern including the Superfund site. The Superfund site includes about 14 miles of the river from the dam at Sheboygan Falls, Wisconsin, east to the harbor on Lake Michigan, including the flood plain of this part of the river. The Area of Concern includes the entire watershed of the Sheboygan River. From 1950 until 1969, the Army Corps of Engineers dredged the lower river and harbor for navigation purposes. The dredging was stopped when heavy metals were found in the sediment. After more testing and sampling, high levels of PCBs also were found. In 1986, the area was added to Superfund's National Priorities List. Three potential sources of contamination were named, and after negotiations, one of the potentially responsible parties agreed to undertake a Remedial Investigation/Feasibility Study (RI/FS) to determine the extent of contamination and look at potential remedial alternatives to deal with the contamination. An engineering firm was hired to conduct the RI/FS. Certain remediation alternatives and associated alternatives are now being assessed including: biological treatment within a pilot confined treatment facility, sediment removal, *in situ* armoring, and monitoring programs.

Greg Goudy of the Michigan Department of Natural Resources presented a summary of the Remedial Action Plan for the Saginaw River and Bay AOC. The Saginaw River empties into Saginaw Bay, located along the eastern shore of Michigan's lower peninsula. As he stated, the water quality of the Bay and River have improved over the last 20 years, but problems still remain. Three primary water quality problems have been recognized in this area. The first is eutrophication which has lead to extensive algal blooms causing taste and odor problems with drinking water from the bay. Second is bacterial contamination caused by combined sewer overflows that discharge raw sewage into the Saginaw River during heavy rains. Finally, there is contamination by anthropogenic compounds such as PCBs and chlorinated dioxins. These have been found in fish tissue and have resulted in public health advisories against fish consumption. The intent of the RAP is to restore the river and bay area to a water quality that is safe so that the areas can once again be used as originally intended without risk to human or environmental health.

Tom Murphy from Environment Canada, discussed the Hamilton Harbour AOC. Hamilton Harbour is located in Hamilton, Ontario on the western bank of Lake Ontario. The main pollutants in the harbour are PAHs, coal tar, and heavy metals. These chemicals have led to the unhealthy fishery, which is of great concern to the public, who have formed a citizens action group. Source controls imposed on the industries in this area have reduced air and water contamination; however, some contaminated "hot spots" still exist. In the areas with low metal contamination, there has been natural degradation of the PAHs and coal tar. Pretreatment methods to make the metals less bioavailable so the bacteria can more easily degrade the PAHs and coal tar are being tested. Another concern, however, is oxygen availability in the sediments. For the biological degradation of PAH compounds, oxygen is necessary; however, the sediments are largely under anoxic conditions. At this time, the recommendation is to dredge and treat the "hot spots" while continuing to study remedial alternatives to this problem.

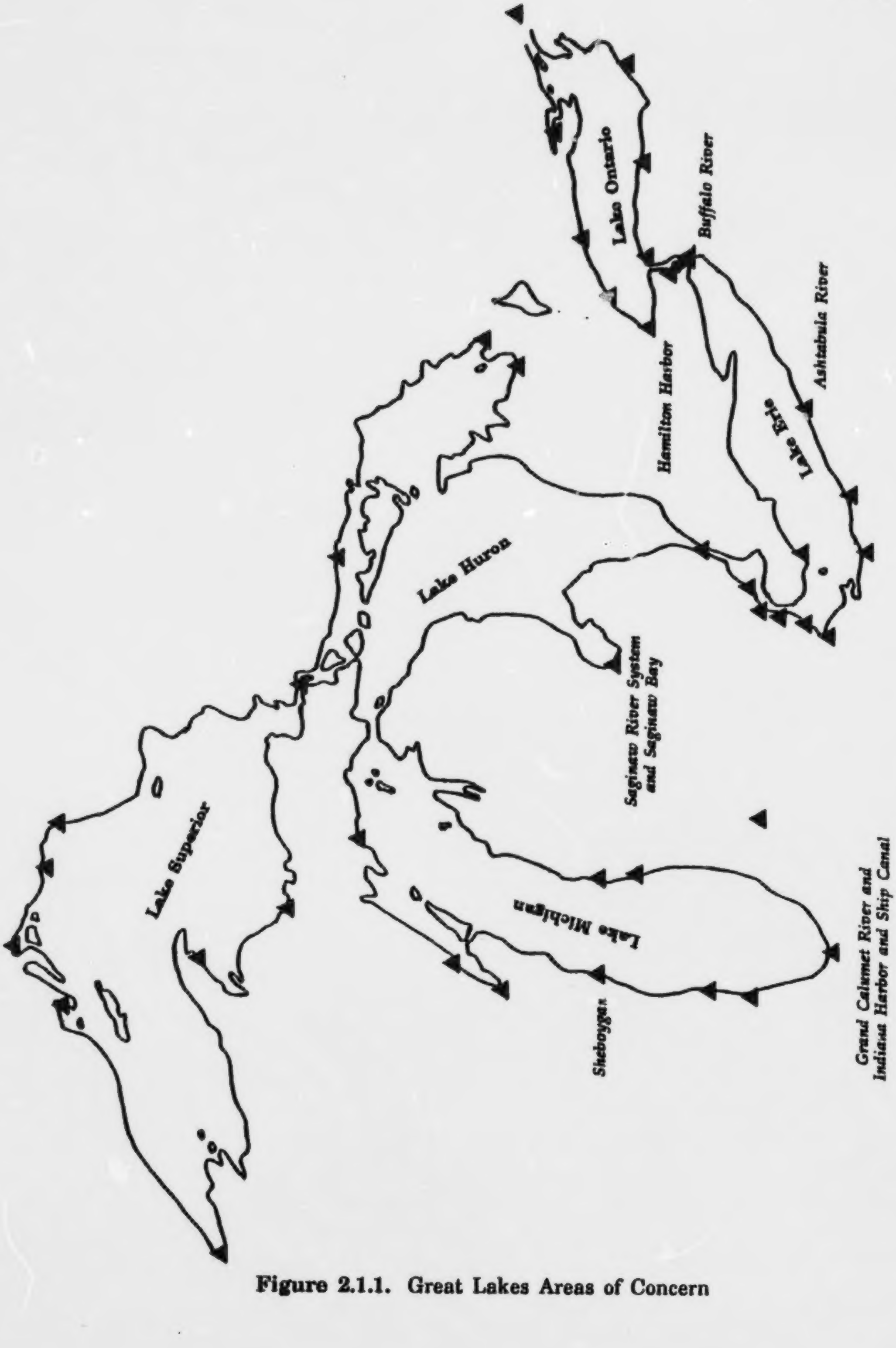
Lake Ontario
Buffalo River
Ashtabula River
Hamilton Harbor
Lake Erie
Lake Huron
Saginaw River System and Saginaw Bay
Lake Superior
Lake Michigan
Sheboygan
Grand Calumet River and Indiana Harbor and Ship Canal

Figure 2.1.1. Great Lakes Areas of Concern

2.2 Polychlorinated Biphenyls

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The congener mixtures of polychlorinated biphenyls (PCBs), produced by Monsanto, were sold under the trade name Aroclor, and contained from 30 to 60 individual congeners (chlorinated analogs of the parent biphenyl of 209 congeners theoretically possible). The last two digits of the number specifying each Aroclor mixture, i.e., 12*48* relate the percent chlorine content by weight of that mixture. Hence, Aroclors 1242 and 1248 are generally referred to as the lower (molecular weight) Aroclors and contain mostly di-, tri-, and tetrachlorobiphenyls, whereas Aroclors 1254 and 1260 are referred to as the higher Aroclors and contain mostly penta-, hexa-, and heptachlorobiphenyls. Recent evidence, much of which was presented during this session, shows that the complete microbial degradation of Aroclors is possible. However, the complexity of the microbial processes responsible for degradation, the complexity of the compounds themselves, and the complexity of sediment interactions with microbes and individual congeners makes this class of compounds one of the greatest challenges to bioremediation technologies.

Ronald Unterman presented information regarding the aerobic biodegradation of PCBs. Under aerobic conditions, PCB biodegradation is a cometabolic process in which another substrate, such as biphenyl, is required as a carbon and energy source. Because no advantage may be gained by the indigenous microorganisms in degrading PCBs (no energy is gained), the introduction of exogenous organisms, specifically isolated for their PCB degrading abilities, may facilitate this process. He noted that Envirogen, Inc. is actively involved in isolating bacterial strains with PCB-degrading capabilities, elucidating the biochemical pathways by which these compounds degrade, and isolating the genes responsible for the various steps involved in this degradation. Only the lower chlorinated congeners (i.e., mono-, di-, tri-, tetra-, and some penta-) are amenable to aerobic degradation. As the number of chlorine substituents increases on the biphenyl moiety, aerobic degradation is reduced. The positional selectivity of PCB-degrading strains was also noted, suggesting that the use of several strains may result in the widest range of degradation of all congeners. Several key parameters that must be evaluated when optimizing aerobic degradation in the field include bioavailability, temperature, and utilization of proper microbial strains. He stressed that experiments purporting to show biodegradation of PCBs by simply quantifying total GC peak areas must be carefully evaluated.

John Quensen presented results of laboratory experiments designed to elucidate the anaerobic biodegradation processes of PCBs. He stressed that anaerobic reductive dechlorination occurs only for the more heavily chlorinated PCB congeners. Several of the mono- and di-chlorinated congeners do not appear to be dechlorinated to any extent and represent terminal products of the higher chlorinated congeners. Reductive dechlorination may be of selective advantage to microorganisms in that it can result in a gain in energy for the organisms and can serve as a terminal electron sink. Terminal electron acceptors are often limiting for microbial growth in anaerobic systems. Drs. Quensen, Boyd, and Tiedje have developed a method of transferring PCB-degrading organisms from acclimated sediment to clean or sterilized sediments. Such transfers of activity have now been made for over 10 serial passes. He discussed the difference in dechlorination patterns within sediments from various locations historically exposed to different Aroclor mixtures. In all studies, however, accumulation of *ortho*-substituted products was observed. The extent of dechlorination was shown to be concentration dependent. This may result both from decreased bioavailability of compound at lower concentrations and/or from increased growth of organisms at higher compound concentrations. He related these studies to the potential for bioremediation of contaminated sites, suggesting either that anaerobic biodegradation alone will reduce sediment toxicity, or that anaerobic/aerobic sequential treatment may reduce the total concentration of PCB congeners. Site assessment should involve evaluation of the presence of dechlorinating microorganisms, *in situ* dechlorination patterns, sediment type, nutrient and organic carbon concentrations, inhibitor concentrations, and the bioavailability of the PCBs.

G-Yull Rhee reported on laboratory studies of the anaerobic dechlorination of Aroclor 1242 and a single congener (2,3,4,2',4',5'-hexachlorobiphenyl) in Hudson River sediment. In the Aroclor 1242 studies, dechlorination patterns were investigated as a function of Aroclor concentration (100 to 1500 ppm on a sediment dry weight basis, and reducing conditions (sulfide-reduced synthetic medium). After 3 months, significant changes in congener patterns were evident, especially at 300 and 500 ppm Aroclor 1242 with mono-, di-, and trichlorobiphenyls comprising 98% of the total remaining PCBs. *Ortho*-substituted congeners showed the most significant increases. After 6 months of incubation, congener profiles for the 100 and 800 ppm concentrations showed significant dechlorination, whereas no difference was observed at 1200 and 1500 ppm. Similar to the results of others, no biodegradation other than dechlorination was found. Anaerobic incubation of the single hexachlorobiphenyl produced congeners with two to five chlorines per molecule. The relative concentration of these products varied with incubation time.

William Sonzogni addressed the issue of whether PCBs are being biologically dechlorinated in the Sheboygan River under ambient conditions. The contamination in this river is believed to be primarily from Aroclor 1248 and 1254. Total PCB concentration ranged from 1586 µg/g downstream from the site of contamination, to 0.04 µg/g upstream from the site, with the highest PCB concentrations found in areas of sediment deposition. He presented strong evidence that biological dechlorination was occurring in the river. This evidence included the following observations: a shift in congener profiles (compared to 1248 and 1254) from the higher chlorinated to the lower chlorinated congeners exists in sediment samples; *meta*- and *para*- chlorinated congeners were depleted more than *ortho*-chlorinated congeners; several specific congeners were found in abundance; and finally congener patterns were found to be PCB-concentration dependent with only samples with greater than 50 µg/g total PCB showing these patterns. The physical and chemical processes that affect congener distribution were also discussed. Abiotic degradation was ruled out because of the extreme conditions (temperature, pH) necessary for this to occur over a reasonable time frame. Similarly, preferential sorption of the more hydrophobic congeners would not result in the observed patterns. Laboratory experiments with river sediments have yet to confirm these patterns. He also reported on a multidimensional gas chromatography technique used to resolve congeners which normally co-elute with conventional gas chromatographic methods. This analytical method is useful in analysis of co-planar PCBs (those with dioxin-like toxic properties). Concentrations of these congeners represent a fractional percentage of Sheboygan River PCBs.

Daniel Abramowicz presented results of laboratory studies in which the rate of anaerobic dechlorination of PCB mixtures was enhanced by the addition of either nutrients, a complex carbon source, a reducing medium, or surfactant Additionally, he presented information regarding the aerobic treatment of Hudson River sediments that had been previously dechlorinated in the environment. The addition of minimal medium to Hudson River sediment slurries was shown to increase the rate and magnitude of anaerobic dechlorination. Addition of trace metals (at concentrations of less than 0.02 ppm) also increased the rate of PCB dechlorination. The addition of the minimal medium and a chemical reducing agent (cysteine hydrochloride) resulted in different patterns of dechlorination, indicating growth of different microbial populations. Dechlorination was shown to occur in numerous, aged PCB-contaminated sediments, including those from the Hudson River, the South Glens Falls dragstrip (amended with Hudson River sediment), and Woods Pond. Aerobic treatment of Hudson river sediments that had previously undergone extensive dechlorination of the higher-chlorinated congeners (>85% mono- and dichlorobiphenyl remaining) resulted in greater than

70% reduction of PCB concentration after one day of treatment.

Dawn Foster reported on the Sheboygan River and Harbor Remedial Investigation/ Feasibility Study Program. In the first phase of this program, the contaminants of concern were identified to include PCBs and eight metals. This investigation led Tecumseh Products Company (one of three potentially responsible parties) to propose an Alternative Specific Remedial Investigation, which consists of pilot-scale studies to investigate various bioremediation alternatives and bench-scale studies to investigate other alternatives. The primary objectives included: evaluation of the potential to enhance biodegradation within a confined treatment facility (CTF); evaluation of *in situ* armoring and the anaerobic biodegradation of PCBs associated with these capped sediments; evaluation of mechanical dredging methods and monitoring of the impact of these activities on the water column; and bench-scale tests of other innovative technologies. The pilot-scale CTF constructed for the enhancement studies has a capacity of 2500 cubic yards and has four cells that can be used to test various treatment scenarios. In addition, various schemes will be examined for the treatment of the cell effluent. Bench-scale studies are currently underway at the University of Michigan that will provide information for the design of CTF enhancement studies by the addition of various amendments. Armoring of in-place sediments was accomplished by placing a geotextile material over the in-stream sediments followed by successive layers of bank run off material (6 inches), another geotextile layer, and a final layer of stones and gabions. Sampling ports through these layers will allow for the monitoring of the natural biodegradation process.

Questions and comments during the PCB discussion sessions encompassed a number of issues; some related and others very specific and unique. The topics dealt with in some detail, in order of their deliberation, included the following.

Development of a Sediment Testing Protocol. The speakers described many laboratory experiments which all have a common theme - that of measuring biodegradation of PCB compounds in sediment systems, and amending these systems to enhance rates of transformation. However, no standardized testing protocol exists to facilitate testing by other scientists or engineers for assessing the feasibility of bioremediation at other sites. It was suggested that such a protocol be developed, and could be used as a guideline, as opposed to a methods document, simply because of the continuously developing nature of this science, and the rapidly expanding data base. It was mentioned that the EPA's Biosystems Technology Development Program is currently developing a testing protocol for contaminated aerobic soils, and that much could be learned from this other effort in developing one for PCB-contaminated sediments. Such a document would be of value to Regional (Superfund) scientists and engineers who must evaluate and oversee bench-scale and pilot-scale studies, and to Remedial Action Plan coordinators who must develop remedial options for contaminated sites.

Deposition of Other Contaminated Sediments on Armored Material. Several questions were asked concerning the integrity of the armored sediments and/or the possibility of re-sedimentation of other contaminated sediments on the armored areas, necessitating re-armoring of the Sheboygan sediments. In response, the pros and cons of armoring were discussed. Basically, armoring can only be evaluated as an option in areas where (1) dredging of sediments is not necessary, and (2) high currents will not disturb the armoring material. In the case of the Sheboygan sediments, re-sedimentation of contaminated sediments should not occur because of the elimination of the source (basically, the sediments are the current source).

Bioaccumulation of PCBs in Lower Organisms. It was asked whether the trends in bioaccumulation of PCBs in lower organisms should coincide with those found in higher organisms (i.e., fish). The discussion that followed addressed the issues of both chemical phase distribution and chemical metabolism. From a thermodynamic standpoint, the potential to bioaccumulate (normalized to organism lipid content) in higher and lower organisms is the same. Factors limiting the kinetic uptake and depuration of these compounds in these organisms, however, may differ. In addition, the ability of some organisms to metabolize these compounds may result in body burdens less than those found in other organisms that can not metabolize them. The thermodynamic potential, the limiting kinetic factors (including such things as migratory patterns), and the organism's ability to metabolize the compounds must all be factored into the observed environmental bioaccumulation of these compounds.

Natural Substrates of the Aerobic Pathways of PCB Degradation. Because the aerobic degradation of PCBs occurs through a cometabolic pathway, a question was raised concerning the identity of the natural substrates for which this metabolic pathway exists, and the natural distribution of the organisms containing the responsible enzymes. The point was raised that, in natural systems, either the concentration of the final electron acceptor and/or carbon source is sometimes the growth limiting factor, not the energy source, *per se*. It was suggested that diagenetic humic material, which contains a considerable amount of aromatic structure and already contains fairly reduced carbon, is the natural substrate. This would account for the relatively ubiquitous distribution of PCB-degrading organisms in the environment.

Mass Balance Accounting of PCBs in the Environment. Part of the problem in identifying natural PCB degradation is that the historical mass loadings of PCBs into various river and harbor systems is not known, and therefore mass balance estimates on losses cannot be easily made. From sampling exercises on the Hudson River between the mid '70s and '80s, it appears that half of the PCBs estimated to be present from the first sampling period (approximately 500,000 lbs) have been lost from the system. It was suggested that long-term sampling programs be initiated in areas where physical transport mechanisms are minimized and where good mass balances can be measured to get a better idea of the extent to which natural biological decay processes are occurring. Such studies may be possible using existing confined disposal facilities.

Effects of Toxic Metals on PCB Degradation Rates. In most of the Areas of Concern, when PCBs are present, heavy metal contamination coexists to some extent. Very little information is available, however, concerning the toxicity of various metal species to PCB degraders. Also, it should not be assumed that high concentrations of metals will decrease degradation rates or are responsible for low degradation rates. Speciation and redox state is important, as well as how the metals are associated with the sediment material. It was generally agreed that metal toxicity should be addressed to some extent in bench-scale studies as metal toxicity will be very site-specific.

Questions of Scale-Up and Number of Pilot Studies. The basic question "where do we go from here" was asked. Do we start new studies, and at what level of effort should these studies proceed (i.e., bench, pilot)? The general consensus of the group seemed to be that currently we are working with a fairly small data base. Several studies have shown positive results, and several have so far been negative. All the effects of, and relationships among, the various controlling factors are not known; hence, the clearest path to site-specific optimization is not always obvious. It was generally agreed that as the results of more studies become available, biological treatment technologies will be refined, and the limits of these technologies will become clearer. Because each level of scale-up involves different aspects of treatability, the design of more pilot-scale studies, based on the results of bench-scale studies was suggested.

Acceptable Clean-Up Concentrations. A concern was raised regarding the fact that there is generally good success at high PCB concentrations (> 50 ppm) and poor success at low concentrations (< 50 ppm): Whereas, even single digit ppm concentrations of PCBs in sediment (dry weight basis) may relate to significant concentrations in fish species. The suggestion was made that engineered systems should focus on these lower concentrations where other chemical or physical destruction technologies do not appear to be economically feasible as final remediation remedies. The point was raised that this phenomenon (concentration dependence) may be largely a consequence of reaction kinetics (including mass transfer limitations) or microbial induction. Both of these causes can be assessed at the bench-scale level.

2.3 Polycyclic Aromatic Hydrocarbons

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Polycyclic aromatic hydrocarbons (PAHs) are a major class of environmental contaminants that are byproducts of 1) burning of fuel, 2) generation of synthetic fuels from fossil fuels, and 3) wood treatment. This class of compounds exhibits a wide range of toxicity, hydrophobicity, and recalcitrance in aquatic systems. While biodegradation of low-molecular-weight PAHs by a wide variety of microorganisms is well documented, there is limited information on the microbial utilization of the more recalcitrant and toxic PAHs consisting of four or more fused rings. In order for bioremediation to be considered a viable treatment of PAH-contaminated sites, the organisms, the processes, and the environmental conditions necessary for the degradation of these compounds must be identified. The speakers in this session address this challenge.

Carl Cerniglia discussed the use of a *Mycobacterium* sp. in the remediation of PAH wastes. The pyrene-degrading bacterium was isolated by direct enrichment from sediment taken from an oil field in Port Aransas, Texas. The bacteria were found to be quite versatile, degrading both low and high-molecular-weight PAHs possessing up to five fused rings. In microcosm studies, the organism was able to compete against bacteria indigenous to a variety of environments (freshwater, marine, pristine, polluted), and enhanced the mineralization of PAHs. He noted that the rates of degradation were dependent on compound structure and site history. Lower-molecular-weight PAHs were degraded faster than higher-molecular PAHs, and contaminated sites (freshwater and estuarine) demonstrated higher degradation rates than pristine ones. Low levels of organic nutrients were reported to be necessary to initiate growth, suggesting the degradation process is co-oxidative. In addition, inorganic nutrient supplements (N and P) enhanced PAH degradation. He pointed out that the mechanism of oxidation is unique as the *Mycobacterium* has both mono-and dioxygenases to catalyze PAH degradation.

H.J. van Veen addressed the problem of contaminated sediments (oil, PAHs, and metals) in the Netherlands. These sediments are of particular concern not only because of their environmental impact, but also because of the need for frequent dredging of the country's many waterways. The speaker gave a survey of the current state of full-scale sediment remediation and the development of biological treatment. Volume reduction of dredged sludge consists of a combination of two techniques: hydrocyclones and dewatering. The "heavier" sand fraction is separated from the finer and often more highly-contaminated fraction using a hydrocyclone, which utilizes tangential flow and centrifugal force. He stressed that this operation will not benefit cleanup of dredged sediment consisting mainly of fine particles or with a high organic carbon content. After separation, the fines fraction is dewatered with a belt press, filter press or decanter using polyelectrolytes. The results of a number of practical cases demonstrate that this type of treatment is fairly successful; however, a couple of problems were pointed out. First, the composition of the sludge often deviates from that expected based on preliminary investigation. Second, in some cases, the sand fraction has high PAH concentrations. When all size fractions are contaminated, a sludge can only be treated intensively in a bioreactor. Whereas sludges that can be fractionated are more effectively treated extensively, i.e. the sand fraction can be land-farmed and the fine fraction can be considered a waste liquid and treated in an aeration basin. Intensively treating PAH-contaminated sediments was shown to be faster than the extensive treatment of the fractionated material; however, over longer time periods both processes were equally effective. He noted that practical considerations, such as material volume, rates of degradation, space and cost will determine whether intensive (bioreactors) or extensive processes are required.

John Rogers presented the work of James Mueller and colleagues on the microbial degradation of PAHs and their relevance to bioremediation. The efforts of this group have been focused on the isolation of microorganisms capable of degrading high-molecular-weight (HMW) PAHs. Mixed bacterial cultures capable of utilizing HMW PAHs as sole sources of carbon and energy for growth have been isolated. He described how they are making use of these microorganisms in a recently developed tri-phasic sequential treatment system for the remediation of creosote and similarly contaminated soil and water. Under a Federal Technology Transfer Act, they were able to transfer some of their biotechnology to an engineering firm which provided separation technology. The steps in this remediation process include conventional soil washing, membrane extraction, and biodegradation of extracted pollutants. Each step in this process results in the volume reduction of contaminated material. Depending on the type of starting material, soil washing can reduce the contaminated volume to as little as 10% of the initial value. While the soil washing process reduces the volume of material requiring treatment, the process generates large amounts of contaminated wash water along with accumulated fine particles. To address this problem, reverse osmosis hyperfiltration through porous stainless steel membranes is applied to dewater and concentrate pollutants. While the effectiveness of this system on soil wash water is currently being evaluated, they have demonstrated that >99% of creosote components present in contaminated groundwater are removed. The speaker emphasized the potential capabilities of the membranes to : 1) fractionate mixtures of chemicals to increase degradation efficiencies or reduce toxicity (e.g. metals), and 2) recycle surfactants used in soil washing. Finally, the wash water is fed to specially enriched microbes housed in continuous flow bioreactors. The ability of these organisms to degrade artificial creosote mixtures has been demonstrated. Field demonstrations of this sequential treatment system are currently being evaluated.

John Glaser discussed the use of white rot fungi (*Phanerochaete chrysoporium* and *P. sodida*) to degrade a variety of target pollutants, including PAHs, in a variety of media. *Phanerochaete sp.* grow quite rapidly on decaying wood. Consequently, this fungus possesses great potential to degrade aromatic components of hazardous waste, based on its ability to degrade lignin. The enzymes of this fungus are extracellular, extremely strong oxidizers, largely non-specific, and not commonly found in other organisms. The speaker pointed out that the non-specificity of these enzymes provides this organism with a capacity to degrade a wide range of substrates (e.g. PAHs, PCBs, pesticides, and dyes). Two types of media have been recently tested, liquid treatment using rotating contactors, and soil treatment. The liquid treatment shows promise and is currently under pilot-scale evaluation to better control pH and the mixing domain within the reactor. The application of wood chips inoculated with *Phanerochaete chrysosporium* and *P. sodida* to soil contaminated with pentachlorophenol resulted in 82% and 85% reduction, respectively, after 46 days. He noted that this fungus does not grow naturally in soil and is non-pathenogenic to plants and animals. The required field conditions (e.g. target compound and oxygen levels, temperature, reactor configuration) for optimal biodegrading activities are currently being investigated.

2.4 Metals

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To summarize the metals session, a brief overview of the Bureau of Mines and the related areas of research it is involved with are given. This is followed by a summary of the session presentations which addressed the research ongoing at the Bureau of Mines and associated research at the Department of Energy's Idaho National Engineering Laboratory (INEL) related to this area and the possible application of this research to the remediation of inorganic-contaminated sediments. The presenters stressed that all the remediation answers to metal-contaminated sediments do not currently exist, but rather that some interesting possibilities in this area, analogous to other current ongoing research in the field of mining and metallurgy, show potential applicability.

The Bureau of Mines was established in 1910 as a Federal Agency in the Department of the Interior. The Bureau is a relatively compact and mature agency by Washington standards. The Bureau employs 2,200 people and is organized into three main directorates: Finance and Management, Information and Analysis, and Research. The research component of the Bureau is the largest element of the Bureau's overall program, employing about 1,300 people, with nine dedicated laboratories located across the country. The Bureau is different from most Federal agencies in that the Bureau performs its research *in house* instead of contracting it out: the one exception to the inhouse research is a healthy program in concert with the Department of Energy's Idaho National Engineering Laboratory (INEL), where two of the sessions speakers (Arpad Torma and Peter Pryfogle) are employed. Bureau of Mines research is targeted at three main areas: (1) Health, Safety, and Mining Technology, (2) Minerals and Materials Science, and (3) Environmental Technology. The Bureau is responsible for a number of major activities related to the minerals industry. Among these responsibilities is the performance of research on mining and metallurgical technologies. This research has led to a number of major developments that have benefitted the industry and the people of this country.

The 75 years of research and technical experience have also resulted in the Bureau becoming the government's principal expert in the area of selective extraction of inorganic ions. This includes technology to extract low concentrations of metals and other inorganic materials from their host environment, solid or liquid. This capability includes another relatively new technique: "biotechnology", which is the use of bacteria to treat metal-contaminated solids and liquids. The "newness" really refers to the use of biotreatment, *under controlled conditions*, as part of a metallurgical treatment process; nature has employed this approach for millions of years. These mechanisms have been and are being employed in the minerals industry on a daily basis as part of leaching operations, for example, for the production of copper. Bacteria were enhancing copper leaching long before man was aware of the bacterial leaching interaction. This same basic mechanism, operating on an uncontrolled basis, contributes to acid drainage from coal mines. Leaching inorganics from solids can be enhanced using bacteria and, alternatively, other types of bacteria can precipitate metals and destroy toxic inorganic processing chemicals in solutions. Both aerobic and anaerobic microorganisms are involved in these processes. This biotechnology can be applied beyond the minerals industry to the field of Superfund and RCRA remediation. Biotechnology often produces a lower level of contaminants in the treated material than is possible to achieve using conventional physical and chemical treatments. In some cases, combinations of biotechnical, chemical, and beneficiation techniques might be the only way to achieve the low level of contaminants in treated materials required by environmental legislation.

Almost half of the Bureau researchers are involved in research that can be generally described as "metallurgical" in nature. Research on extractive processes -- selective capture of one or more elements from host materials that are either natural or recycled materials -- represents a large component of this part of the Bureau's program. Four of the nine Bureau of Mines laboratories have ongoing projects involving bioextraction of metals and INEL is actively involved in associated biotechnical research.

In the first presentation, I presented the Salt Lake City Research Center's work involving the bioaccumulation of elements such as arsenic, cadmium, lead, mercury and selenium, from solution using both viable bacteria and biomass immobilized in what we call "BIOFIX" beads. In addition, the destruction of cyanide in process streams using viable bacteria was discussed. This research may have direct application to inorganics removal from sediment-associated waters. This research is being expanded at the Salt Lake Research Center to include bioleaching of inorganic contaminants from sediments and mine tailings using bacteria. The nature of these low-level, high-volume wastes makes most processing options extremely expensive. Bacterial leaching *in situ* or on heap pads may provide an answer to this widespread problem.

Hank Edenborn, from the Pittsburgh Research Center, reported on biotechnology for the remediation of acid mine drainage from coal mines. He described the use of "wetlands" technologies for this purpose, and how this technology may be directly applicable to sediment remediation. He also described the use of bactericides to inhibit bacterial leaching in the event that sediments should have to be dredged from waterways immediately, but could not be treated for a period of time. Bactericides would prevent the biologically mobilized inorganic contaminants from leaching from the sediments and entering the surface or groundwater during storage prior to treatment.

Betty Baglin reported on research at the Reno Research Center on the bacterial leaching of manganese, platinum and gold ores as a means of improved leaching technology. She related the applicability of this work to remediation of contaminated sediments.

The Department of Energy's Idaho National Engineering Laboratory (INEL) has been studying the mechanisms of bacterial metals removal from solids and the application of these results in conjunction with the Bureau of Mines. Arpad Torma from INEL discussed biochemical possibilities of inorganic sediment remediation and Peter Pryfogle provided information on INEL's research capabilities.

Robert Lambeth from the Spokane Research Center presented information on linking biological and hydrogeochemical mechanisms (models) of sediment leaching. This is a complex research area and involves (1) field and laboratory data requirements, and (2) computer model requirements. The Bureau's Spokane Research Center has been using geochemical computer models to interpret hydrogeochemical mechanisms of mine tailings and sediment leaching. Recently, personnel from the Spokane and Salt Lake City Research Centers conducted a joint sampling trip to a copper-gold tailings impoundment in Washington State in the hope of linking biological to hydrogeochemical mechanisms of inorganic leaching. Currently a "cookbook" for predicting contaminant fate at new sites does not exist, but rather the presentation focused on an approach to developing techniques for predicting contaminant fate at new sites based upon knowledge gained from sites that have already been studied.

The research presented during this session and described in the abstracts has great potential for biotreatment of inorganics in sediments. Successful development of the biotechnical techniques may provide on-the-shelf technology for environmental problems untreatable with conventional technology today.

2.5 Conclusions

During the past decade, a great deal has been learned regarding biological processes that act to transform or mineralize anthropogenic pollutants, including those discussed in detail during the Workshop. The ability of microorganisms to degrade or transform chlorinated organic compounds such as the PCBs, polycyclic aromatic hydrocarbons (PAHs), and metal species is now well documented. Yet, an understanding of how these mechanisms function in environmental systems, to the extent that we can consistently optimize them for bioremediation purpose, is not totally understood. Two general areas in which information gaps can be grouped for the problem at hand include: (1) The specific processes and mechanisms controlling observed degradation rates and patterns, and (2) issues associated with extrapolation of bench-scale studies to pilot or full scale field studies. A majority of the specific questions and issues that were discussed during the workshop fell into these two areas.

Clearly, a significant amount of information on the biological transformations of pollutants already is known from process research. Much of this research is at the phenomenological level. The results have helped identify empirically, or allude to mechanistically, the interactions among microorganisms, pollutants, and the sedimentary and aqueous media in which they exist. These interactions can be rather complex, even for rather simple systems, such as the transformation of a single compound by a pure microbial culture in an homogeneous solution. In this simple system, characterization of the degradation process requires an understanding of nutrient and growth requirements, the kinetics of transformation reactions, degradation pathways, pollutant concentration dependencies, effects of alternative substrates and electron acceptors, temperature dependencies, the effects of metabolic inhibitors, and in some cases, the effects of varying carbon sources. The additional complexity associated with investigating the same microbial decay process in natural or manipulated sediments is obvious. Additional consideration must be given to organic and inorganic inhibitor availability, combined inhibitory effects, pollutant bioavailability and the kinetics of this availability, and microorganism competition or cooperation of the indigenous bacteria. Although a complete understanding of how these processes interact at specific sites would result in the most obvious approaches to treatability, a comprehensive understanding may not always be necessary. In many cases, biological treatment efficiency may be significantly enhanced (above background levels) by regulating a few critical factors limiting activity. These factors must be identified at the bench-scale level through simple process studies. In many cases, differences in these controlling factors are reasons for the site (or sediment) specific nature of biological treatability successes. Clearly, while much is known, a better definition of the chemical, physical, and biological processes (or factors) controlling observed transformation rates and pathways in natural and manipulated sediments will enhance the frequency and degree of bioremediation successes.

On the other hand, the extrapolation of results from bench-scale studies to pilot or full scale studies is largely untested for remediation of sediments contaminated with the pollutants of concern. Examples of extrapolation were presented during the Workshop. They include technologies developed for the separation or removal of metal species from mine tailings or drainage, and other bioremediation technologies evolving the remediation of soils or liquid waste streams containing organic contaminates. Also, applied bioremediation may take many forms, from simple low energy *in situ* (in place or CDF) systems to highly engineered, high energy systems. Each form has its own list of design factors or parameters that must be considered when optimizing treatment. As more field-scale efforts become realities, however, systems obviously will be refined, and a clearer connection between bench-scale methods (and treatment efficiencies) and applied field scale processes will become evident.

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ABSTRACTS

3 AREAS OF CONCERN

3.1 Buffalo River Remedial Action Plan Strategy

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Abstract

In February 1987, the Buffalo River Citizens Committee was formed to assist the New York State Department of Environmental Conservation in the preparation of a Remedial Action Plan (RAP) for the Buffalo River. The goal of the plan is to restore and maintain the chemical, physical, and biological integrity of the Buffalo River ecosystem in accordance with the Great Lakes Water Quality Agreement (GLWQA). The GLWQA lists conditions that indicate impairments of environmental quality. Scientific data and professional opinions were used to confirm the impairments and link them to causes. The RAP addresses the river's environmental concerns through a remedial action strategy to address contaminants and their sources in the Buffalo River.

Introduction

As a tributary to the Great Lakes, the largest freshwater basin in the world, the Buffalo River watershed feeds one of the most important ecosystems in New York State. Conditions that impact the water quality of the Buffalo River may affect the water quality of the downstream international waters of the Niagara River, Lake Ontario, and the St. Lawrence River. As a result, pollutants added to the Buffalo River ecosystem may contribute to impairments of these downstream waters that are part of the Great Lakes system. Improvements to the environmental integrity; of the Great Lakes can best start with its harbors and tributaries, such as the Buffalo River, where pollutants are concentrated before they disperse throughout the lakes.

The high concentration, of past industrial discharges to the Buffalo River has polluted the river and its sediments. The area exhibits environmental degradation and some beneficial uses of water and biota are impaired.

The United States-Canada International Joint Commission (IJC) designated the Buffalo River as one of 42 Areas of Concern (AOC) where pollution problems may affect the health of the Great Lakes ecosystem. The IJC requested that the responsible jurisdiction prepare plans for remediation of the AOCs.

The 1987 amendments to the United States-Canada Great Lakes Water Quality Agreement (GLWQA) specify requirements for "remedial action plans" (RAPs) for the Areas of Concern. The RAPs are to define environmental problems and identify actions needed to restore beneficial uses of the waterbody. Plans are to embody a systematic, comprehensive, ecosystem approach to restoring and protecting the biota and water quality. They should set time schedules, name responsible agencies, and describe processes to monitor the AOC environment and track implementation. The lead agency for a RAP should work closely with citizens to develop an ecosystem-based plan that represents the concerns of the local community.

The Buffalo River RAP was developed by the New York State Department of Environmental Conservation (DEC) in cooperation with citizens concerned about the river's revitalization. In 1987 a group of interested citizens was appointed by DEC as the Buffalo River Citizens' Committee (BRCC) comprising 21 environmental, small business, university, community, and local government representatives. BRCC representatives and key DEC staff created a 10-member steering committee that directed the development of the Buffalo River RAP. The steering committee established the goals of the RAP, mapped out a project workplan, defined responsibilities, and developed and reviewed data summaries and document drafts.

This document summarizes the Buffalo River Remedial Action Plan that resulted from this cooperative endeavor. More detailed information about problems and sources affecting the Buffalo River, remediation programs, recommendations, and agency commitments is contained in the full RAP report.

Setting

To understand the problems of the Buffalo River and the remedial actions needed to resolve these problems, it is important to understand several things about the river: (1) where it is located and the general character of its surroundings (the geography); (2) the uses of the river from which benefits are derived (beneficial uses); (3) the occurrence, distribution, and movement of water (hydrology) and sediments in the AOC and its watershed that carry pollutants and constrain remedial actions; and (4) the water quality of the three tributary creeks that drain into and affect the AOC.

The following describes the Buffalo River AOC and watershed area and sets the scene for the discussion of remedial actions. It describes geography, beneficial uses, and hydrology and bottom sediments, first for the AOC, and second for the watershed. A description of the water quality in the tributaries is also included.

AREA OF CONCERN

Geography

The Buffalo River AOC is located in the City of Buffalo, Erie County, in Western New York State (Figure 3.1.1). It extends about six miles from the mouth of the Buffalo River to the eastern border of the City of Buffalo. In this area, the water level of the river is influenced by the level in Lake Erie. The river flows from the east and enters Lake Erie near the head of the Niagara River.

The river is dredged to just below the junction of Cazenovia Creek, and is used as a transportation channel. It passes through an industrial area characterized by some active industries, but also by many abandoned buildings, junkyards, and trash-littered areas that give it the appearance of an industrial wasteland.

Beneficial Uses

Industrial

The Buffalo River historically served the industries along its banks as a convenient transportation corridor, a source of process and cooling water, and a receptacle for wastewater. The major industries include two grain milling firms, General Mills and Pillsbury, two chemical companies, Buffalo Color and PVS Chemicals (both formerly Allied Chemical), coke and steel manufacturing by Donner-Hanna Coke and Republic Steel (both no longer operating), and a Mobil Oil Company refinery (currently functioning only as a storage terminal). The Buffalo River Improvement Corporation (BRIC) was formed in the late 1960s to supply water from the Buffalo Harbor on Lake Erie to these industries (except for the grain milling firms) for process and cooling purposes. Because of industrial plant closures and process shutdowns, current BRIC pumpage and discharge is down from 120 million gallons per day in the late 1960s to 18 million gallons per day.

Currently industries are operating under strict pollution control regulation by the state. However, their past operations have created a legacy of contaminated sediments on the river bottom and abandoned hazardous waste deposits along its banks.

Combined Sewer Overflows (CSOs)

The City of Buffalo discharges excess water collected during times of runoff through a combined sewer overflow system. The system was designed to collect and transport both sanitary sewage and wet-weather storm flow in the Buffalo area. CSOs prevent sewers from backing up and flooding city streets during storms. However, their existence also means untreated sewage is discharged during some storms and this has been a problem in the AOC. The Buffalo Sewer Authority currently is reevaluating the CSO system.

Commercial Shipping

The US Army Corps of Engineers maintains the river within the AOC (by periodic dredging) as a transportation corridor for commercial freight vessels. Dredging disturbs bottom life and the bulkheading and dock construction by private interests along the river bank have removed wetlands and shallow areas which were once habitat for fish and wildlife.

Recreation

People use the AOC for recreation. A few people fish the river, although the state health department advises against consuming certain species of fish taken there. Fishing use is reduced also because of limited land access points, a perception that the river is polluted, and the ready availability of nearby alternative fishing sites. Small, powerboats travel the river in the AOC for recreational purposes primarily near the mouth or the river.

Swimming is not a common activity, probably because Lake Erie is more accessible and more aesthetically pleasing.

River Hydrology and Bottom Sediments

The US Army Corps of Engineers dredges the river to maintain it at a depth of 22 feet below low lake level for navigation purposes. Dredging the Buffalo River slows the river flow and increases the volume of backflow from Lake Erie. When the flow is high, the river has a "riverine" (one directional) character. Under low flow conditions, the river takes on an "estuarine" (two directional) character. When this occurs, the river is influenced by lake level variations associated with the passage of storms through Lake Erie and by seasonal thermal differences between lake and river waters. The river and lake waters do not remain separate, but mix at varying rates depending on relative water temperatures.

Studies of bottom sediments show that the river traps all sand particles until its flow exceeds 20,000 cubic feet per second, which occurs only rarely. The finer clay and silt particles pass through the river during the high flows associated with most storms, but are retained during periods of normal and lower flow. The wider portions of the river trap the most particles.

In addition to natural river flow, the river is augmented by water pumped from Lake Erie by BRIC.

WATERSHED

Geography and Beneficial Uses

The watershed of the Buffalo River has a drainage area of 446 square miles and is fed by three tributaries: Cazenovia Creek, Buffalo Creek, and Cayuga Creek.

Wastewater Discharges: Industrial, Municipal

Our society is still dependent on waterbodies as receptacles for treated industrial and municipal waste. Cayuga, Buffalo, and Cazenovia Creeks receive treated discharges from a number of industries and municipal treatment plants, as well as sewer system overflows. The quality of the Buffalo River is influenced by the flow from these tributaries.

Land Uses: Agricultural, Woodland, Residential

Farmland and wooded areas dominate the upland areas of Cayuga Creek and the land areas adjacent to Buffalo Creek and Cazenovia Creek. Several park and recreational areas are located along these waterways.

The lower reaches of Cayuga Creek pass through residential communities, as do parts of Buffalo and Cazenovia Creeks. Along their way to the Buffalo River, these creeks receive runoff from agricultural, suburban, and urban lands that contains sediments and pollutants picked up from city street, farms, and from rain and snow. The magnitude of this pollutant load and its effect on the Buffalo River are not known.

Recreation

The Buffalo River drainage basin supports a variety of fish habitats. Conditions range from brook trout habitat in some upper streams to warm water species habitat in the lower, urban areas. To enhance recreational opportunity, DEC stocks trout and pan fish. Salmon, black bass, and northern pike are among the many species found in the Buffalo River and its tributaries.

Watershed Hydrology and Current Water Quality

The three major tributaries of the Buffalo River are generally fast-flowing streams with many rapids and low waterfalls that serve to aerate the water. Water quality monitoring stations on the three tributaries show high water quality. Comparison with Class A standards (the best use is classified as drinking water) indicates that the three tributaries meet the established standards for all conventional parameters and metals except iron. In addition, analysis of volatile organic compounds in 1987 revealed virtually no volatile organics, further indicating a high quality of water in these streams.

THE RAP GOALS AND THE PLANNING PROCESS

The goals for remediation were identified at the beginning of the process jointly by DEC and BRCC.

Short Term Goal

The short-term goal of the Buffalo River RAP is to restore and maintain the chemical, physical, and biological integrity of the Buffalo River ecosystem in accordance with the GLWQA. To meet this goal, this plan takes steps toward the restoration of water quality which provides for propagation of fish, shellfish, and wildlife, and for recreation in and on the water, consistent with state law, rules, and regulations as they continue to evolve.

This goal is called "short-term" because, given a funding commitment, it could likely be accomplished within 15 years.

Long Term Goal

The long-term goal is to eliminate the discharge of pollutants to the Buffalo River. This includes, but goes beyond, the GLWQA policy of the virtual elimination of discharges of persistent toxic substances.

The immediate intent of this RAP is to address the short-term goal. As remedial action moves toward the short-tenn goal, the long-term goal will also be approached. In addition, the various statewide program activities driving New York State toward pollution elimination, such as technology-based discharge permit limits, will continue to operate. Because these are statewide activities, the Buffalo River RAP includes them in the plan by reference only. The RAP focuses on the immediate objective - attainment of the short-term goal, through actions specific to the Buffalo River.

Ways of Determining if the Short-Term Goal is Being Met

NYS Stream Classification

Impairments to the short-term goal are ultimately determined by criteria derived from the NYS stream classification system, which classifies every waterbody in New York State according to the public's desired "best use" of the water resource. The classification takes into account such factors as the character of bordering lands, stream flow, water quality, and present, past, and desired future uses of the water. after a formal public participation process, including public hearings, DEC assigns to each fresh surface waterbody one of the following classifications. Each class includes all the best uses for classes below it.

Class	Best Use
AA, A	Drinking Water
B	Primary Contact Recreation
C	Fishing and Fish Propagation
D	Fishing

Each designated classification has a set of standards defining the type and quantity of substances the water can contain and still be used as intended. Classifications are subject to review every three years. Public input is an important part of this process. The Buffalo River is currently classified D. Proposals to change that classification are under consideration by DEC in its statewide review of water classifications. The Buffalo River Citizens' Committee has requested a change to a B classification.

Great Lakes Water Quality Agreement

The GLWQA (Annex 2) lists 14 impairment indicators to be examined by the RAP process. These are presented in Table 3.1.1. For the Buffalo River, those indicators that relate to the best use of fishing (class D) are the ones that are important for determining whether or not impairments exist. These GLWQA indicators are: restrictions on fish and wildlife consumption, tainting of fish and wildlife flavor, degradation of fish and wildlife populations, fish tumors or other deformities, bird or animal deformities or reproduction problems, degradation of benthos, eutrophication or undesirable algae, degradation of aesthetics, degradation of phytoplankton and zooplankton populations, and loss of fish and wildlife habitat.

If the waters were classified as B (best use swimming) the additional GLWQA impairment indicator "beach closings" would be also used. If the waters were classified as a (best use drinking water supply) then the GLWQA impairment indicator "restrictions on drinking water consumption, or taste and odor problems" would be used.

TABLE 3.1.1
GREAT LAKES WATER QUALITY AGREEMENT IMPAIRMENT INDICATORS

(i) Restrictions on fish and wildlife consumption;
(ii) Tainting of fish and wildlife flavor;
(iii) Degradation of fish and wildlife populations;
(iv) Fish tumors or other deformities;
(v) Bird or animal deformities or reproduction problems;
(vi) Degradation of benthos;
(vii) Restrictions on dredging activities;
(viii) Eutrophication of undesirable algae;
(ix) Restrictions on drinking water consumption, or taste and odor problems;
(x) Beach closings;
(xi) Degradation of aesthetics;
(xii) Added costs to agriculture or industry;
(xiii) Degradation of phytoplankton and zooplankton populations; and
(xiv) Loss of fish and wildlife habitat.

Two GLWQA impairment indicators are anomalous because they do not have any counterpart in the New York State classification system. These are: restrictions on dredging activities, and added costs to agriculture or industry.

The RAP addresses all 14 indicators, but the overall impairment is related to the best uses according to New York State's stream classification system.

RAP STRUCTURE

The process of developing the RAP proceeded as follows:

- Identify goals
- Assess Impairments - The short-term goal is addressed by examining information on water quality, sediments, and aquatic life that shows whether or not the best uses are impaired. The 14 specific indicators provided by the GLWQA helped determine these impairments. The impairments are determined by the New York State stream classification system.
- Identify Pollutants or Disturbances - When an impairment indicator suggests an impairment, all available information is examined to determine the cause of the impairment. In some cases, definite causes cannot be assigned with a high degree of certainty.
- Identify Sources of Pollutants or Disturbances - The points of entry of pollutants or the origin of disturbances are determined.
- Describe Remediation Strategy and Commitments - The overall remedial strategy identifies actions to address the sources of pollutants and disturbances causing impairments. Where information is not sufficient to recommend remedial action, the strategy identifies investigations needed to obtain this information.
- Describe Monitoring Program - Measurements and examinations of the ecosystem reveal whether or not the remedial actions work as planned, and whether or not the indicators of use impairment show recovery.
- Describe Tracking - Progress reports and periodic RAP updates, both with participation of the concerned public, provide a process for tracking plan implementation.

Impairments, Causes and Sources

The Buffalo River and its sediments have been polluted by past industrial and municipal discharge and disposal of waste. Fishing and survival of aquatic life within the Area of Concern have been impaired by PCBs, chlordane, and polynuclear aromatic hydrocarbons (PAHs). Fish and wildlife habitats have been degraded by navigational dredging of the river and by bulkheading and other alterations of the shoreline. Low dissolved oxygen and DDT are likely causes of aquatic life degradation, but they have not yet been definitely established as such. In addition, metals and cyanides in the sediment prevent open lake disposal of bottom sediments dredged from the river.

Contaminated bottom sediments are the one certain source of pollutants causing impairments. Other sources have been identified as potential sources because the pollutants causing impairments are known to exist at these locations, but the link between the source and the impairment has not been clearly established. The potential sources include inactive hazardous waste sites, combined sewer overflows, and other point and nonpoint sources of pollution. A summary of impairments, causes and sources is shown in Table 3.1.2.

Remedial Objectives and Recommendations

A comprehensive and focused strategy has been developed to:

- remediate the bottom sediments;
- establish a river monitoring program that will determine whether potential sources contribute to impairments;
- continue the on-going programs that remediate inactive hazardous waste sites, control point source discharges, and manage nonpoint sources; and
- improve fish and wildlife habitat.

The recommended program is:

Remediate Bottom Sediments

Objective:
Correct the impairments to the Buffalo River's fishery and aquatic life caused by contaminated sediments.
Recommendation:

1. Develop a model of sediment flow and deposition in the Buffalo River in order to determine the potential for armoring layers to be established over the contaminated sediments in certain sections of the river.
2. Develop sediment criteria that will allow decisions to be made about which particular bottom sediments are causing impairment of the fishery and aquatic life.
3. Assess the river sediments based on criteria to determine specific areas of the river where remedial work is needed.
4. Evaluate removal/armoring alternatives and then carry out appropriate remedial work.

Improve Stream Quality Monitoring

Objective :
Ensure that all sources have been addressed in the remedial action plan.
Recommendation:

1. Establish an automated sampling station on the Buffalo River so that the amounts of contaminants of concern can be accurately determined.
2. Develop models to relate amounts of contaminants in the river to their potential for harming fish or aquatic life.

Objective:
Determine whether low dissolved oxygen in the Buffalo River is likely to impair the fishery.
Recommendation:
Carry out an intensive dissolved oxygen study.

Remediate Inactive Hazardous Waste Sites

Objective:
Prevent inactive hazardous waste sites from contributing contaminants to the river.
Recommendation:
Continue the on-going program for remedial work in the Buffalo River drainage area with particular attention to protecting the Buffalo River itself.

Remediate Other Nonpoint Sources as Necessary

Objective:
Prevent the nonpoint sources from adversely affecting the river. [Nonpoint sources are sources that do not discharge to the river at well-defined points such as through a pipe.]

Recommendation:
1. Use stream water quality monitoring to determine whether or not these sources are making a significant contribution to the amount of pollutants in the river.
2. If nonpoint sources are important, determine which ones require remedial action.
3. Select and carry out appropriate control or remedial actions.

Maintain Controls on Municipal and Industrial Wastewater Facilities

Objective:
Insure that municipal and industrial point sources do not significantly contribute to impairment of the fishery or aquatic life. [Point sources are sources that discharge to the river at well-defined points, such as through a pipe.]

Recommendation:
1. Renew permits, as they expire, incorporating current technology and water quality based limits.
2. Carry out monitoring of industrial and municipal discharges and compliance or enforcement actions as needed.

Improve Combined Sewer Overflow Systems

Objective:
Insure that combined sewer overflows do not significantly contribute to impairment of the fishery or aquatic life. [Combined sewer overflows are used to relieve the flow to sewage treatment plants during storms when surface runoff would cause the flow in the sewers to exceed the capacity of the system.]

Recommendation:
1. Carry out system modeling to determine where improvements can be made to increase flow within the system and minimize overflow.
2. Design and carry out improvements as necessary.

Remediate Other Point Sources as Necessary

Objective:
Insure that other point sources do not significantly contribute to impairment of the fishery or aquatic life.

Recommendation:
1. If stream water quality shows that other point sources are likely to be a problem, then identify these sources.
2. Design and carry out remedial work as required.

Restore Fish and Wildlife Habitat

Objective:
Improve fish and wildlife habitat in and along the river.

Recommendation:
1. Carry out an assessment of habitat conditions and the potential for improvement in the Area of Concern.
2. Develop a habitat improvement plan.
3. Acquire the necessary land.
4. Design and carry out specific habitat improvement projects.

Commitments and Future Actions

The Department of Environmental Conservation has committed to a number of initial actions in this plan where funding is available. All of these initial actions are to be completed in 1990. As further funding becomes available, further commitments can be made. DEC has made commitments for specific actions to begin the remediation strategy:

REMEDIAL ACTION COMMITMENTS

A. Stream Water Quality Monitoring

1. *Establish a flow-activated sampling station*
   DEC will have a station in place in 1990 that will allow sample collection to be correlated with flow, so that loadings of chemicals transported by the river can be measured. The next step will be to collect water quality samples from this station and in the upper basin and compare the results to determine loadings from sources along the river.

2. *Carry out comprehensive dissolved oxygen measurements on the Buffalo River*
   DEC will carry out dissolved oxygen measurements on the Buffalo River to determine whether lack of dissolved oxygen is impairing best uses and, if it is, the causes of decreased dissolved oxygen. The next step, if needed, will be to propose remedial actions.

B. Bottom Sediments

1. *Develop requirements for a sediment model improvement*
   DEC will develop the requirements for a model that will allow prediction of scouring and deposition. The next step will be to contract, develop, and implement the model.

2. *Develop methods to determine sediment criteria*
   DEC will urge EPA to develop national sediment criteria. Criteria should relate directly to environmental effects of sediment so decisions can be made on the need for remedial work. The next step will be to apply the criteria to the sediments in the Buffalo River in order to map the portions of the river that are contributing to use impairments.

C. Inactive Hazardous Waste Sites

1. *Conduct Phase I site investigations*
   DEC will continue Phase I investigations for each site in the Buffalo River Basin. All Phase I studies will be completed in 1990. The next step will be to conduct phase II investigations.

2. *Conduct Phase II site investigations*
   DEC will conduct nine Phase II investigations. The next step will be to prepare and conduct Remedial Investigation/Feasibility Studies at these sites when required.

3. *Conduct Remedial Investigation/Feasibility Studies (RI/FS)*
   DEC will conduct two RI/FS at hazardous waste sites. These studies will be completed in 1990. The next step will be to design remedial measures at these sites.

D. Municipal and Industrial Wastewater Facilities

Continue discharge permit monitoring
DEC will continue this ongoing program for all permitted discharges. Permits will be

reissued every five years based on current technology requirements and water quality standards.

E. Combined Sewer Overflows

Evaluate the combined sewer model
BSA is responsible under its State Pollutant Discharge Elimination System permit for developing and evaluating the model of their CSO system. This work is underway and is expected to be completed in 1990. The next step will be to use the model to simulate alternatives for minimizing overflows. Then, remedial measures will be planned based on the model simulation results.

F. Fish and Wildlife Habitat

Develop a plan for assessment of habitat conditions
DEC will develop a plan for the assessment of habitat conditions and improvement potential by March, 1990. The next step will be to carry out the assessment according to the plan

A continuing process, based on annual status reports and workplans, has been established for reporting on remedial progress, for making commitments as funding becomes available, and for revising the remedial action plan as new information develops.

TABLE 3.1.2
Summary of Impairments, Causes and Sources

No.	Impairments and Impairment Indicators	Impairments	Likely Causes	Known Sources	Potential Sources
1.	Restrictions of fish and wildlife consumption	Yes	Polychlorinated biphenyls	Bottom Sediments	Inactive hazardous waste sites
			Chlordane		Bottom sediments
2.	Tainting of fish and wildlife flavor	Likely	Polynuclear aromatic hydrocarbons	Bottom sediments	Inactive hazardous waste sites Combined sewer overflows
3.	Degradation of fish & wildlife populations	Likely	Low dissolved oxygen[1]		Bottom sediments Inactive hazardous waste sites Combined Sewer overflows Other point sources Other nonpoint sources
4.	Fish tumors and other deformities	Yes	Polynuclear aromatic hydrocarbons	Bottom sediments	Inactive hazardous waste sites Combined sewer overflows
5.	Bird or animal deformities or reproduction	Likely	Polychlorinated biphenyls	Bottom sediments	Inactive hazardous waste sites
			DDT and metabolites		Bottom sediments
6.	Degradation of benthos	Yes	None Identified	Not applicable	Not applicable (N/A)
7.	Restrictions on dredging activities	Yes	Metals and cyanides	Bottom sediments	Inactive hazardous waste sites Combined sewer overflows Other nonpoint sites Other point sites
8.	Eutrophication or undesirable algae	No	N/A	N/A	N/A
9.	Restrictions on drinking water consumption or taste and odor problems	No	N/A	N/A	N/A
10.	Beach closings	No	N/A	N/A	N/A
11.	Degradation of aesthetics applicable	No	N/A	N/A	N/A
12.	Added costs to agriculture or applicable industry	No	N/A	N/A	N/A
13.	Degradation of phytoplankton & applicable zooplankton population	No	N/A	N/A	N/A
14.	Loss of fish and wildlife habitat	Yes	Physical disturbances	Bulkheading Dredging Steep bank slopes	

[1] River channelization is also a potential factor

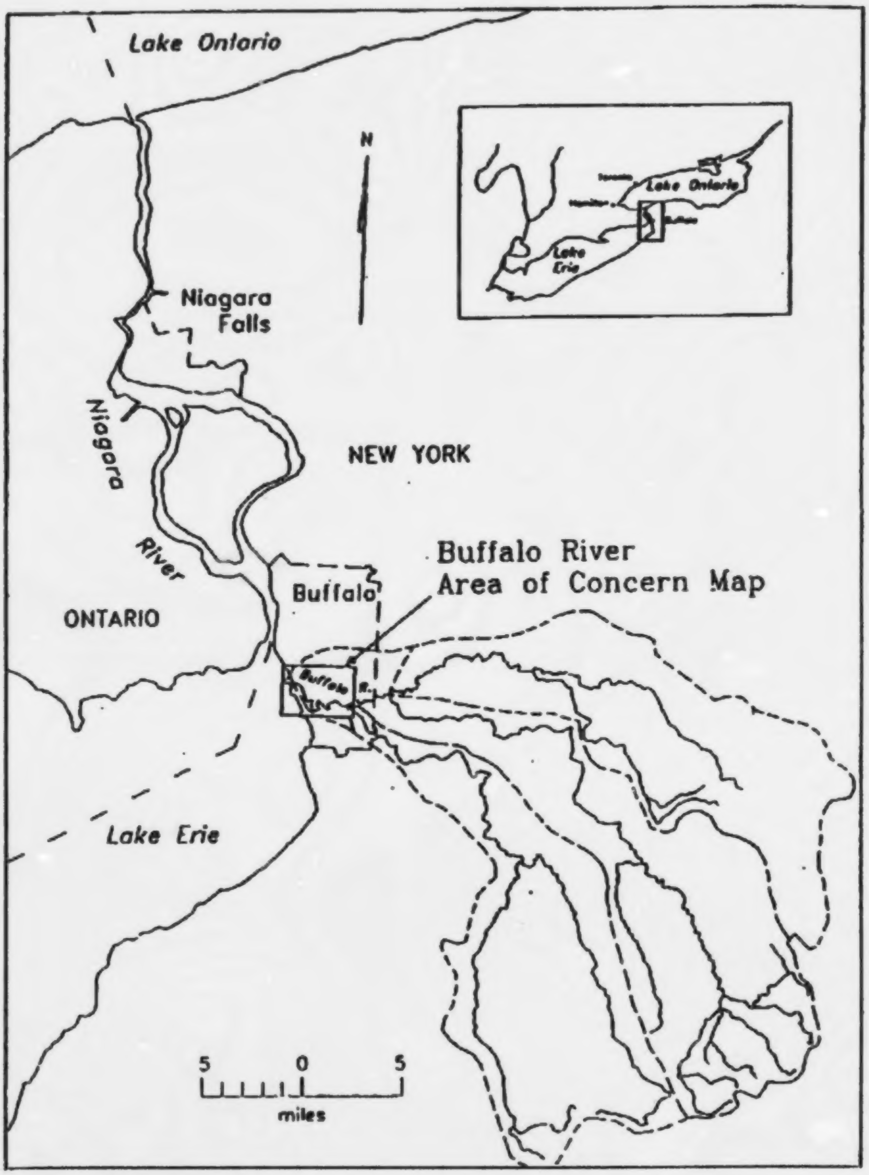
Lake Ontario
N
Lake Ontario
Lake Erie
Niagara Falls
Niagara River
NEW YORK
Buffalo River Area of Concern Map
Buffalo
ONTARIO
Buffalo R.
Lake Erie
5 0 5
miles

Figure 3.1.1. Buffalo river area of concern location map

3.2 Fields Brook Superfund Site/Ashtabula River Area of Concern

Peter Sanders
U.S. EPA
230 South Dearborn Street
Chicago, Illinois 60604

The Ashtabula River and Fields Brook are located in extreme northeast Ohio, in Ashtabula County, approximately 55 miles east of Cleveland, Ohio (Figure 3.2.1). The Ashtabula River drainage basin covers an area of approximately 137 square miles. The drainage basin is predominantly rural and agricultural, with the city of Ashtabula as the only significant urbanized area. The major tributaries include Fields Brook, Hubbard Run, and Ashtabula Creek. Most of the industrial development is concentrated around Fields Brook.

Fields Brook drains a 5.6 mile watershed (defined as the Superfund Site), including areas of Ashtabula Township and the City of Ashtabula (Figure 3.2.2). The brook flows westerly through an industrial area that is considered one of the largest and most diversified concentrations of chemical plants in Ohio, then through a residential area in the City of Ashtabula, to its confluence with the Ashtabula River. The Ashtabula River empties into Lake Erie about 8,000 feet downstream of its confluence with Fields Brook.

Industrial sources have contaminated the sediment in Fields Brook with a variety of organic and heavy metal pollutants (Table 3.2.1), consisting of numerous chlorinated compounds including polychlorinated biphenyls, hexachlorobenzene, hexachlorobutadiene, 1,1,2,2,-trichloroethane, and tetrachloroethene and inorganics including mercury, zinc, arsenic, chromium, cadmium, and lead.

The Fields Brook site was included on the October 23, 1981 Interim Priority List and then placed on the first National Priorities List on September 8, 1983. In March of 1985 the U.S. EPA published a Remedial Investigation (RI) Report for the site and July of 1986 published the Feasibility Study (FS) describing the remedial alternatives considered for site cleanup. A Record of Decision (ROD) was signed by the U.S. EPA on September 30, 1986, which described the selected alternative for the Sediment Operable Unit, which consisted of excavation of contaminated sediments from the brook, temporary storage and dewatering and the thermal treatment of a portion, approximately 16,000 cubic yards, and the solidification and landfilling of the remainder, approximately 36,000 cubic yards, and subsequent water treatment. The volume of the material to be thermally treated verses that which will be solidified and landfilled is based on three factors 1) mobility of contaminants, 2) toxicity and concentration and 3) PCB concentrations. The partition coefficient (K_{oc}) of compounds were considered when determining mobility and the sediment ingestion rate represents a factor that provides a means of quantitative accounting of both toxicity and concentration. A plot of volume of sediments exceeding the 10^{-6} risk guideline verses the mobility was developed. It was determined that for locations that have compounds with K_{oc} values lower than 2,400 ml/g and where sediment ingestion risk associated with the presence of these compounds is greater than the 10^{-6} level, these sediments would be thermally treated. In addition, sediments containing greater than 50 parts per million PCB will be thermally treated. The ROD also proposed two subsequent activities, including a RI/FS to identify any ongoing sources of contamination to Fields Brook and a study to address the contamination in Ashtabula River. On March 22, 1989, the U.S. EPA issued a Unilateral Administrative order to nineteen Potentially Responsible Parties (PRPs) to perform the Sediment Operable Unit Remedial Design activities and the RI/FS to identify sources of contamination. To date six PRPs have agreed to comply with this order. On September 26, 1989, the U.S. EPA and Ohio EPA and five PRPs signed a Consent Order for the PRPs to perform an investigative study of the Ashtabula River. In addition to the objectives outlined under the Superfund ROD, the River Investigation was also conducted to generate data to be used by the U.S. Army Corps of Engineers (COE) to design a dredging program for the river.

The Ashtabula River Investigation, which began in late 1989, includes collection and analysis of sediment, water and fish samples. To date, only results from the sediment sampling have been made available to the U.S. EPA for review. Samples were collected at a total of 115 locations, consisting of two locations in Lake Erie, two locations in Ashtabula Harbor, 103 locations along the main stem of the river and eight locations off the main stem of the river. Samples were collected from sediments in the river using a boat mounted vibrocore rig and in the harbor and lake using a Ponar dredge where vibrocoring was impractical or unsuccessful. Over 450 sediment samples were analyzed, results for compounds included in the "U.S. EPA Guidelines to Classify Sediments From Great Lakes Harbors" are summarized in Table 3.2.2. In general, contamination in the river sediments is greatest below -8 Lake Erie low water datum (LWD; elevation 568.6 feet above MSL at Father Point, Quebec). The COE has tentatively developed a plan to dredge material from the navigation channel to a depth of -6 LWD with an estimated volume of 18,000 cubic yards. This material will be disposed of in a confined disposal area. It has been estimated that nearly 500,000 cubic yards of sediment would have to be dredged from the Ashtabula River Area of Concern for proper clean up.

Work has begun at the Fields Brook Site on Phase I of the Source Control RI. This work involves characterization of the regional ground water basin including piezometer installation, geophysical surveys, soil borings and determination of ground water recharge and discharge reaches; soil gas surveys to help determine the existence and extent of volatile organic compounds (VOCs) in the soil ground water; and industrial outfall sampling, including both dry and wet weather sampling. After Phase I is completed objectives of the RI will concentrate on property or source-specific investigations. Upon completion of the RI, a FS will be carried out to identify potential treatment technologies, pre-screen these technologies and assemble alternatives for detailed analysis which will result in a determination by the U.S. EPA and OEPA of a recommended alternative.

The Sediment Operable Unit design investigation will begin soon, this investigation has been divided into five task investigations (Figure 3.2.3) which will culminate in the final design. The task investigations include:

1. A sediment quantification investigation to better define the volume of sediments to be handled by thermal treatment or solidification;

2. a thermal treatment design investigation involving several test burns (pilot scale), evaluation of the characteristics of the ash generated and identification of Applicable or Relevant and Appropriate Requirements (ARARs) for emissions and residues;

3. a solidification design investigation to develop measurements of treatment effectiveness, guidelines for performance monitoring, refined estimates of the landfill capacity requirements and identify ARARs;

4. a sediment dewatering and wastewater treatment design investigation to evaluate the physical and chemical characteristics of aqueous waste streams that could require treatment prior to discharge and to determine the relative "dewaterablility" of sediments that will be needed for thermal treatment and solidification; and

5. a facility design investigation to identify potential sites for the dewatering, solidification, thermal treatment facility, RCRA-type landfill, and temporary storage facility.

Currently, these five task investigations are scheduled to be completed by early 1992 and results will be used to develop the final design for the Sediment Operable Unit.

Table 3.2.1.
Priority Pollutants Found in Sediment at the Fields Brook Site

Volatiles

Benzene (C)
Chlorobenzene
1,1,1-Trichloroethane
1,1,2-Trichloroethane
1,1,2,2-Tetrachloroethane
Chloroform (C)
1,1-Dichloroethene (C) (I)
Trans-1,2-dichloroethene
Ethylbenzene
Methylene Chloride (C) (I)
Tetrachloroethene (C)
Toluene
Trichloroethene (C)
Vinyl Chloride (C)

Acids

2-Chlorophenol
Phenol

Pesticides

Heptachlor (C)
γ-Hexachlorocyclohexane (C)
α-Hexachlorocyclohexane (C)
PCB 1016 (C)
PCB 1242 (C
PCB 1248 (C)
PCB 1254 (C)

Metals

Antimony
Arsenic (C)
Beryllium (C) (W)
Cadmium (C) (W)
Chromium (C) (W)
Copper
Cyanide
Lead
Mercury
Nickel (C) (W)
Selenium
Silver
Thallium
Zinc

Base/Neutrals

Acenaphthene
Benzidine (C) (I)
1,2,4-Trichlorobenzene
Hexachlorobenzene (C)
Hexachloroethane (C)
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
Fluoranthene
Hexachlorobutadiene (C)
Isophorone
Naphthalene
Nitrobenzene
N-nitrosodiphenylamine (C)
Bis(2-ethyl hexyl) phthalate
Butylbenzyl phthalate
Di-n-butyl phthalate
Diethyl phthalate
Dimethyl phthalate
Benzo(a)anthracene
Benzo(a)pyrene (C)
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Chrysene
Acenaphthylene
Anthracene
Benzo(ghi)perylene
Fluorene
Phenanthrene
Dibenzo(a,h)anthracene
Indeno(1,2,3-cd) pyrene
Pyrene

C = Carcinogenic.
W = Carcinogenic based on human occupational exposure.
I = Carcinogenic based on animal inhalation studies.

Table 3.2.2

ARI - Main Stem River Sediment Samples
Selected Parameters - Statistical Data
Presented of Dry Weight Basis

(Locations 12201 through 20502)

COMPOUND NAME	UNITS	NO.OF SAMP.	NO. OF DET. SAMP.	AVE. CONC.	MIN. CONC.	MAX. CONC.
Arsenic	mg/kg	129	129	812.92	4.46	31.06
Barium	mg/kg	129	129	402.33	35.39	2152.00
Cadmium	mg/kg	129	129	2.76	0.00	25.00
Chromium	mg/kg	129	129	402.81	12.43	5739.91
Copper	mg/kg	129	129	44.26	14.42	414.02
Iron	mg/kg	58	58	30201.37	18441.56	48387.10
Lead	mg/kg	129	129	60.30	9.89	248.06
Manganese	mg/kg	58	58	491.38	124.40	2900.43
Mercury	mg/kg	129	129	0.96	0.00	11.32
Nickel	mg/kg	129	129	41.28	13.61	142.00
Zinc	mg/kg	129	129	209.64	62.47	1161.18
PCB's	mg/kg	400	321	11.85	0.00	660.07

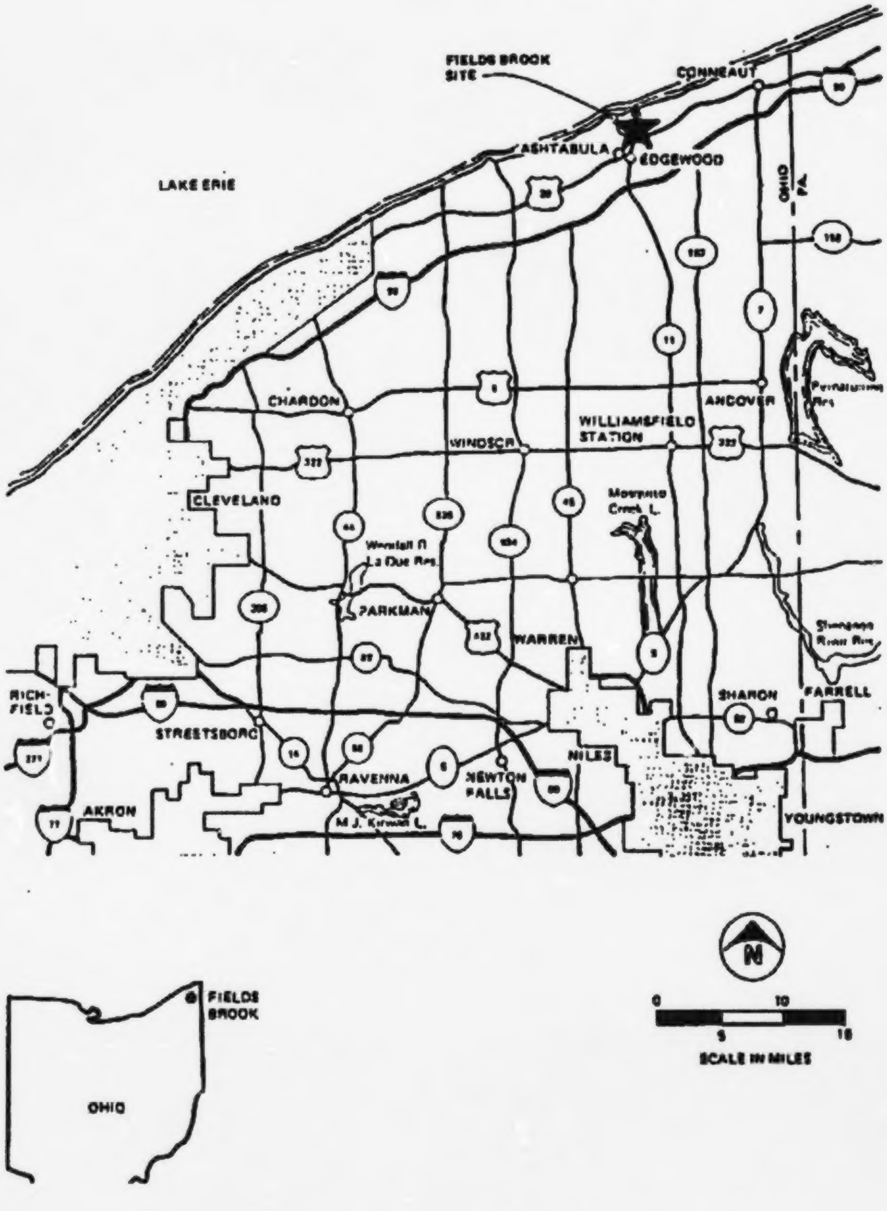
FIELDS BROOK SITE
CONNEAUT
ASHTABULA
EDGEWOOD
LAKE ERIE
OHIO
PA.
90
20
193
7
11
6
CHARDON
ANDOVER
Pymatuning Res.
WILLIAMSFIELD STATION
WINDSOR
322
CLEVELAND
44
528
534
45
Mosquito Creek L.
Wendell R. La Due Res.
306
PARKMAN
422
WARREN
Shenango River Res.
87
5
RICH-FIELD
80
SHARON
FARRELL
62
STREETSBORO
14
88
RAVENNA
NEWTON FALLS
NILES
271
77
AKRON
M J. Kirwan L.
76
YOUNGSTOWN
N
0
10
5
15
SCALE IN MILES
FIELDS BROOK
OHIO

Figure 3.2.1. Vicinity map, Fields Brook

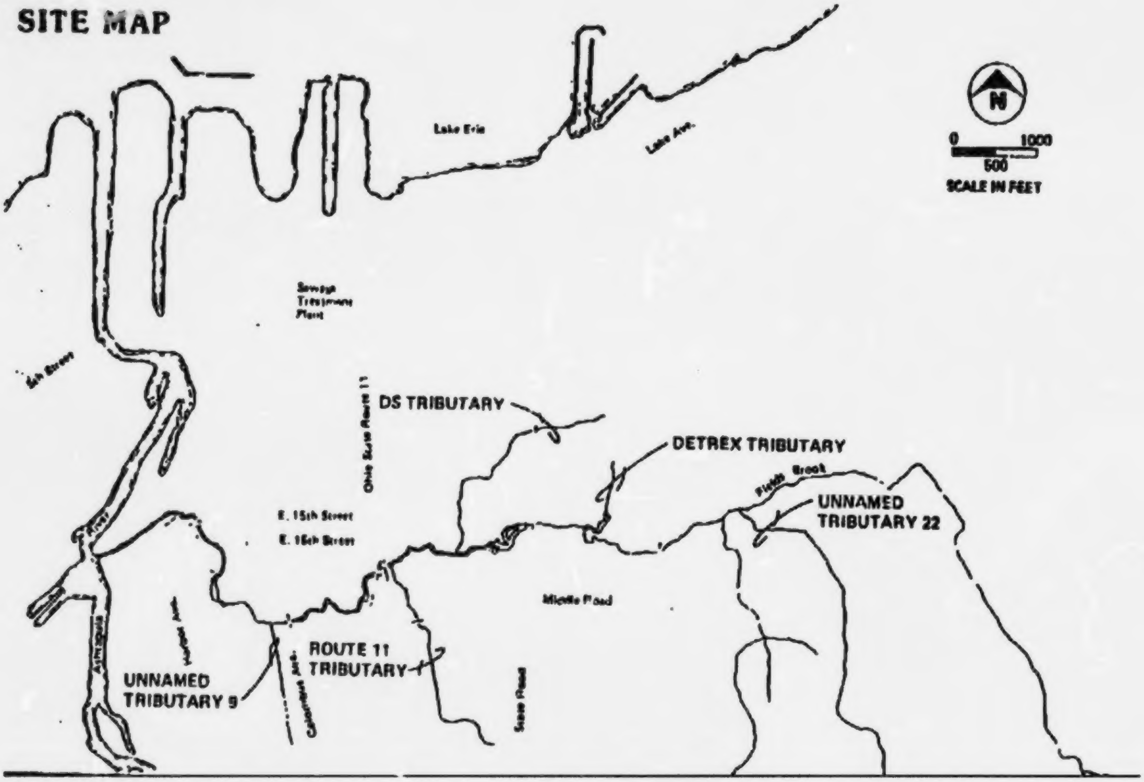
SITE MAP
Lake Erie
Lake Ave.
N
0
1000
500
SCALE IN FEET
Sewage Treatment Plant
5th Street
Ohio State Route 11
DS TRIBUTARY
DETREX TRIBUTARY
Fields Brook
UNNAMED TRIBUTARY 22
E. 15th Street
E. 16th Street
River
Middle Road
Ashtabula
Hubbard Ave.
Columbus Ave.
State Road
ROUTE 11 TRIBUTARY
UNNAMED TRIBUTARY 9

Figure 3.2.2. Fields Brook site map

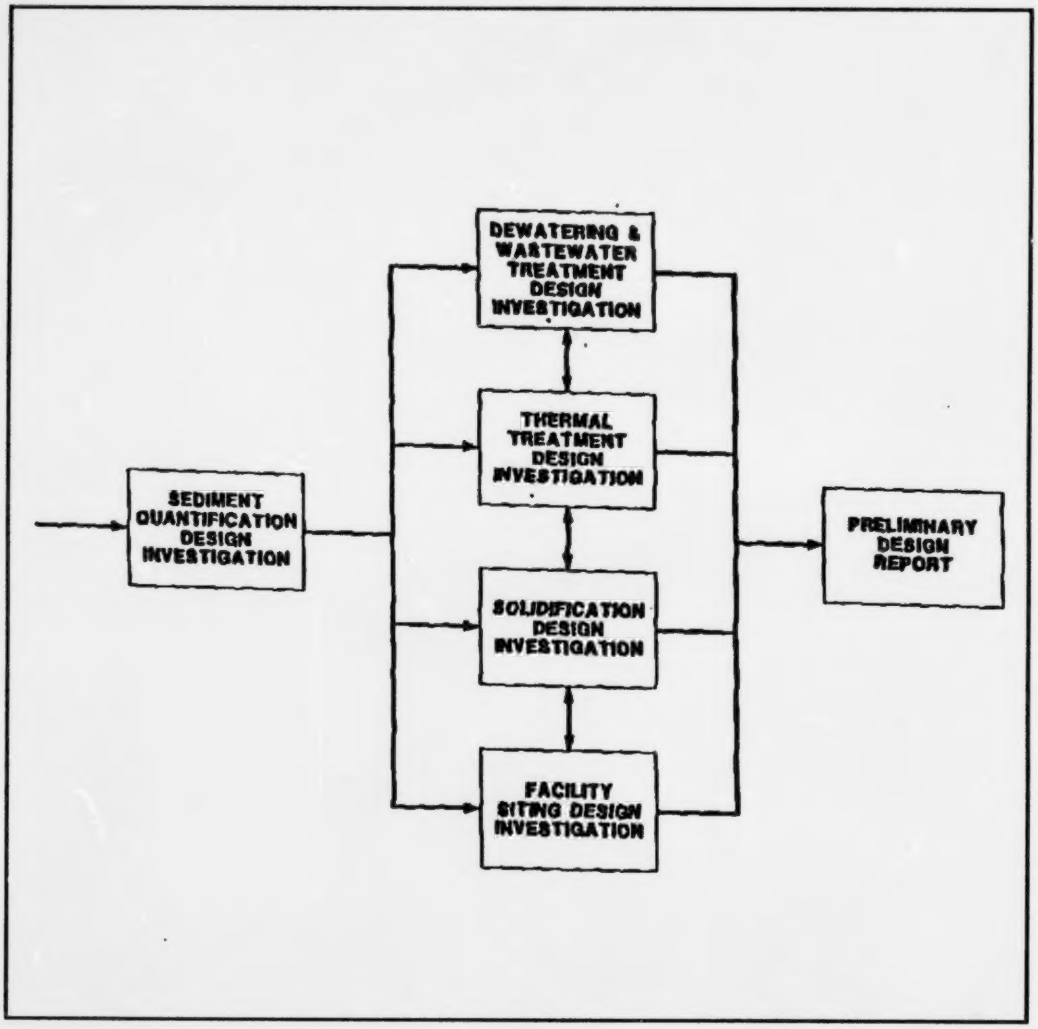
SEDIMENT QUANTIFICATION DESIGN INVESTIGATION
DEWATERING & WASTEWATER TREATMENT DESIGN INVESTIGATION
THERMAL TREATMENT DESIGN INVESTIGATION
SOLIDIFICATION DESIGN INVESTIGATION
FACILITY SITING DESIGN INVESTIGATION
PRELIMINARY DESIGN REPORT

Figure 3.2.3. Sediment operable unit investigations

3.3 Coal Tar Contamination Near Randle Reef, Hamilton Harbor

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Abstract

To support the remedial action plan of Hamilton Harbor, and to determine the extent of coal tar contamination in a toxic area of the harbor, 81 sediment cores were collected for chemical and biological study. Approximately 55,000 m^3 of sediments bounded by Randle Reef, pier 15, and Stelco are contaminated with coal tar. The coal tar distribution is variable but the highest concentrations are near the Stelco outfall pipe. The total concentration of the 16 polynuclear aromatic hydrocarbons (PAHs) in 48,3000 m^3 of near-surface sediments exceeds 200 Íg/g. The concentration of PAHs that results in the death of 50% of *Daphnia magna* and *Hexagenia* is less than 244 Íg/g and 329 Íg/g, respectively. Sediments containing more than 89 Íg/g of PAHs suppress at least half of the photoactivity of *Photobacterium phosphoreum*. The acute toxicity of the sediments of all of Hamilton Harbor is significantly correlated to the PAH concentration.

Management Perspective

Recommendations

A. Needing immediate action.

1. Adopt the following cleanup standard; the mean concentration of PAHs in sediments resulting in the death of 50% of *Daphnia* and *Hexagenia*, and the suppression of 50% of the photoactivity of *Photobacterium* (200 Íg/g).
2. Use the best available safety procedures when handling the most contaminated sediments.
3. Develop a cleanup protocol that includes advanced processing of the most contaminated sediments, i.e., recycling, pyrolysis, but not a simple CDF.
4. Examine existing MOE data to confirm that industrial PAH discharges into combined sewers will not continue to result in the formation of contaminated sediments.
5. Expand upon the current limited data set to confirm that PCBs are not a major contaminant in the sediments of the hot spot.

B. Needing future action.

1. Determine the environmental variables restricting bacterial degradation of the PAHs in Hamilton Harbor.

2. Develop a "finger print" assay to distinguish between coal tar and coal dust.
3. Determine if the black sediments at the northwest corner of Stelco contain high concentrations of coal dust.
4. Determine the relative contribution of coal tar and coal dust to the elevated PAH concentrations in the deep basin of Hamilton Harbor.
5. Determine the relative bioavailability of PAHs in coal tar and coal dust.
6. Determine the effect of coal tar and coal dust on the distribution of benthic invertebrates.

3.3 Advancement Towards A Remedial Action Plan for the Indiana Harbor and Canal, the Grand Calumet River, and the Nearshore Lake Michigan

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-Alas- Indiana is making rapid advancement towards a Remedial Action Plan (RAP) for the Indiana Harbor Canal, the Grand Calumet River, and the nearshore Lake Michigan.

Today, Indiana is preparing Stage One of the RAP. Stage One of the RAP process, in brief, is an identification of the problem. We have committed to the completion of Stage One on or before January 1, 1991. As of today, we're on schedule, and we will meet our target date. Although we have only committed to Stage One of the RAP this year, we are rapidly advancing towards implementation.

Before I discuss the progress toward implementation, it is important for you to understand the scope of the problems we are confronted with in this International Area of Concern.

The Indiana Harbor and Canal and the Grand Calumet River are located about 20 miles southeast of Chicago, Illinois, in the northwestern most part of the State of Indiana. The Area of Concern is commonly referred to as 'The Region'.

This Region produces more steel than any other region of comparable size in the United States, with five active steel companies. It also contains four oil refineries, six crude oil pipelines and 18 refined petroleum product companies.

Located within the Region are five Superfund Sites, 56 CERCLA Sites, 425\RCRA Sites, 23 TSDs, 9 hazardous waste landfills or surface impoundments, and 462 registered underground storage tanks, 150 of which are reported to be leaking.

The Region is currently classified as non attainment of National Ambient Air Quality Standards for particulate matter, ozone, carbon monoxide and sulfur dioxide.

The Region has major groundwater contamination from the petroleum companies and steel industries. Often, because of the regions high water table, contaminated groundwater becomes surface water and thus, causes large oil slicks to appear in the river and harbor.

Regarding surface waters, during the last 20 years considerable improvement has been noted in the water quality of the river and the harbor. In the early 1960s a TV documentary about the river and the harbor was entitled "Too Thick to Navigate, too Thin to Cultivate". The documentary described how the river often caught fire because of the thick layer of petroleum on the surface. Until the 1970's, not even algae lived in the River.

Although water quality has improved, there is much to be done. It is estimated that each year 11 billion gallons of untreated wastewater enters the river and harbor through combined sewer overflows.

Fish communities in the river and harbor are depressed. A combination of lack of food resources, low dissolved oxygen, and toxic stress have resulted in a lack of a stable resident fish community. The quality of biological habitat is poor. The aquatic community is adversely impacted by both organic pollution and toxic stress. Water quality monitoring data have shown problems in these waters with several parameters including ammonia, dissolved oxygen, total phosphorus, chlorides, fluorides, sulfates, oil and grease, bacteria, cyanide, iron, lead, copper, mercury and PCBs.

The 1990 Fish Advisory states that no fish should be eaten from the waters of the Grand Calumet River and the Indiana Harbor Canal.

An important environmental concern is a significant accumulation of contaminated sediments in the river and harbor. Today a three-mile footprint of contaminated sediment stretches into Lake Michigan from the Indiana Harbor. Infrared photos show water intake pipes for the cities of Hammond, Whiting and East Chicago are within 1/2 mile of the sediments. This means that there exists a *potential* threat to the drinking water supplies of approximately 291,000 area residents.

The U.S. Army Corps of Engineers estimates that the cost of removing and *treating* the sediments in the Harbor alone could be as much as one billion dollars. The cost of removing and *treating* the sediments in the Grand Calumet River could be another billion dollars.

The cost of dredging and *storing* the sediments is estimated to cost much less...about 127 million dollars. The issue of where to dispose of the sediments lingers.

The magnitude of the environmental problems in the Region is staggering. But now, let's look at what the new administration in Indiana is doing to address the many problems:

Water Quality Standards

Very significant to the overall success of the RAP is the adoption of the new Water Quality Standards. Previously, the river and harbor were designated for 'industrial' use. A few months ago, Indiana Governor Evan Bayh, signed into law the most stringent water quality standards in the history of the State. The new standards upgrade the designated use of the river and harbor to 'whole body contact recreation' waters. Although it will be several years before the Indiana Harbor Canal and the Grand Calumet River become safe for whole body contact recreation, the adoption of the standards provide the legal frame-work for repairing the damage that one hundred years of industrialization has done to these waterways.

Control of Air Toxics

Indiana is currently holding public meetings throughout the State seeking public participation as the Department moves toward the adoption of rules to control hazardous air pollutants. These new rules will include provisions to address deposition of air toxics into aquatic ecosystems, such as the Great Lakes.

Indiana intends to enact rules after reauthorization of the Clean Air Act in 1990 or after it is clear that Congress will not reauthorize the Clean Air Act this year.

New Office - More Staff

The Indiana Department of Environmental Management will soon open a new office in the heart of the Area of Concern. (In the past, it was necessary for department staff to travel about 160 miles to the area.) The new office will be complimented by the staffing of 27 environmental engineers and scientists. Seventeen of those positions will be new. The new office looks out over beautiful Lake Michigan with only the smoke stacks of U.S. Steel obstructing the view.

Beefed Up Enforcement

Major advancements are being made toward enforcement of criminal and civil environmental laws. A few months ago, the former Superintendent of the Hammond Sanitary District agreed to plead guilty to four felony counts for having submitted falsified Discharge Monitoring Reports to the State. The former operator has now become State's witness as the investigation begins to broaden. The felony convictions of this wastewater treatment plant operator would be only the tip of the iceberg as more prosecution of environmental offenders advances.

As for civil litigation, the State and the U.S. EPA have court actions pending against almost every major discharger in noncompliance in the Area of Concern.

This summer, the United States Steel Corporation, through a Consent Decree filed in federal court, agreed to:

- Spend at least $25 million to upgrade its wastewater treatment equipment and related facilities.
- Spend $7.5 million to investigate and clean up contaminated sediments on the Grand Calumet River bottom.
- Pay a $1.6 million civil penalty for past water pollution violations.
- Develop a comprehensive management plan by June 30 to treat coke plant wastewater.
- Design a corrective action plan to reduce the amount of ammonia, cyanide and phenols in wastewater discharged from the coke plant.
- Improve overall system to collect and treat wastewater from the steel-making process at the plant.
- Determine the makeup and toxicity of sediments in the riverbed and develop a plan to remove or contain them by September 1995.
- Design a program to reduce the volume of oil and grease discharged from the steel plant.

Many other cases are pending before the court and it is expected several dischargers will soon join in the efforts for remediation.

CARE Committee

For the Remedial Action Plan to be successful, the Plan must come from the community and the community must have a vision of its success. For that reason, Kathy Prosser, the new Commissioner of the Indiana Department of Environmental Management appointed 12 community leaders to the new Citizen's Advisory for the Remediation of the Environment (CARE Committee). The Committee is made up of the three mayors from the Area of Concern, a senior union leader, a senior chamber of commerce official, a senior professor of a local university, a senior petroleum company official, a CEO of a major steel corporation, and three recognized environmental leaders from the community. The CARE Committee is chaired by Commissioner Kathy Prosser.

The mission of the new CARE Committee is to advise the Indiana Department of Environmental Management on the matters relating to environmental and recreational restoration and revitalization of the area in and around the near shore Lake Michigan, the Indiana Harbor Canal and the Grand Calumet River, specifically by:

1. Representing the interests of key organizations and constituents in the development of the Remedial Action Plan.
2. Reviewing chapters of the remedial Action Plan as they are developed.
3. Initiating public education programs to:
    a. develop widespread recognition of pollution as a cause of poor water quality and reduced economic and environmental value in the area; and
    b. promote a sense of responsibility for restoration of the area of concern, acceptance of the remedial measures that are necessary to abate pollution problems, and the motivation to implement these remedial measures.
4. Encouraging and assisting the public in participating in the remedial action planning

process, including the development of a vision for the Area of Concern, RAP goals, objectives, remedial measures, and implementation measures.

5. Developing a strategy for implementing the remedial action recommendations in a deliberate, vigorous, and timely manner and uniting the diverse and necessary interests that are essential for successful implementation.

In fulfilling these responsibilities, the C.A.R.E. Committee is to meet the major objectives of the Remedial Action Plan, to:

1. Develop an approach to reduce toxics from all significant sources, including in-place pollutants, to levels that protect human health.
2. Recommend actions needed to reduce nutrient and sediment loadings to the river, harbor and near shore areas to a level that eliminates unacceptable health risks in the Area of Concern.
3. Recommend actions to protect and rehabilitate shorelands, improve land management, provide for compatible recreational and commercial uses, and develop a framework for a long-term dredge and dredge spoil disposal plan associated with the river, harbor, and near shore areas.
4. Describe the measures necessary to bring about new and protect existing spawning areas, reestablish critical aquatic habitats, and reestablish proper species diversity among fish and other aquatic life.
5. Increase public awareness of the beneficial use potential of the Grand Calumet River, the Indiana Harbor Canal, and the near shore Lake Michigan; and encourage public participation in identification of problems and selection of remedial actions.

Conclusion

Do we have all of the solutions yet? No. Are we trying to find solutions? Indeed we are. Are we making progress? A resounding yes!

There are no easy answers to the many environmental problems we are confronted with in Northwest Indiana. But, alas, Indiana is rapidly advancing towards a Remedial Action Plan for the Indiana Harbor Canal and the Grand Calumet River!

3.5 Saginaw River/Bay AOC

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Background

The Saginaw River and Saginaw Bay have been defined as one of 42 Great Lakes Areas of Concern (AOCs) by the International Joint Commission (IJC) because degraded water quality conditions impair certain beneficial uses for which these waters are designated. Environmental programs have produced substantial improvements in Saginaw River and Saginaw Bay water quality over the past 20 years, but additional efforts are needed to address the remaining problems. The most effective way of dealing with these issues is to design and implement site-specific activities that are tailored to the Saginaw Bay area. This would provide a more focused effort than would be possible solely with statewide or national programs.

Consequently, in July 1986, the Michigan Department of Natural Resources (MDNR) began the development of a Remedial Action Plan (RAP) for the Saginaw River/Bay AOC. The RAP was completed two years later in September 1988 with the additional assistance of a wide variety of local, state and federal groups. The principal participants were the Saginaw Basin Natural Resources Steering Committee, the East Central Michigan Planning and Development Region, and the National Wildlife Federation. The RAP is viewed as an iterative document that will be periodically updated and revised as more data are acquired, remedial measures are implemented, and environmental conditions improve. Currently, a large number of activities identified in the RAP are being implemented and it is anticipated that the RAP will be updated following the completion of these efforts.

Environmental Setting

Saginaw Bay is a large and relatively shallow southwestern extension of Lake Huron located midway along the eastern shore of Michigan's lower peninsula (Figure 3.5.1). The bay is 26 miles wide at its mouth along a line drawn between Au Sable Point and Point Aux Barques at the interface with open Lake Huron. From the midpoint of this transect to the mouth of the Saginaw River the bay is 52 miles in length. The bay's surface area of 1,143 square miles is roughly 5% of Lake Huron's total surface area.

The Saginaw Bay shoreline extends for 149 miles and constricts the bay to a width of 13 miles between Point Lookout and Sand Point, approximately midway along the bay's length. This constriction, along with a broad shoal area between Charity Island and Sand Point, divides the bay into inner and outer halves with equal surface areas. The inner bay is much shallower than the outer bay, having a mean depth of only 15 feet and a maximum depth of 46 feet versus mean and maximum depths of 48 feet and 132 feet, respectively, for the outer bay. Consequently, the outer bay contains about 68% of the total bay volume. The total bay volume of 6.8 cubic miles is about 0.8% of Lake Huron's total volume.

The Saginaw Bay watershed consists of 8,709 square miles, which is about 15% of Michigan's total land area. Twenty-eight rivers, creeks or drains flow directly into Saginaw Bay from three drainage areas - the East Coastal, West Coastal, and Saginaw River basins. The Saginaw River basin is the largest of the three and the largest in Michigan, covering 6,276 square miles, which includes 72% of the total Saginaw Bay watershed. The Saginaw River itself is relatively short, extending only 22 miles to the south from the southern end of Saginaw Bay. Though short, the Saginaw River has a large average flow of over 4,000 ft^3/sec, which is about 75% of the tributary hydraulic input to Saginaw Bay. Most of the Saginaw River flow originates from its four major tributaries - the Cass, Flint, Shiawassee and Tittabawassee rivers - with 50% of the flow coming from the Tittabawassee River. All four rivers converge near the head of the Saginaw River.

Four major urban areas are located within the basin - Flint, Saginaw, Bay City and Midland - along with 90 additional city or village municipalities. Two of the four major urban areas are located directly on the Saginaw River, Bay City at its mouth and Saginaw at the head. Midland and Flint are also located in the Saginaw River watershed on the Tittabawassee and Flint rivers, respectively.

The physical boundaries of the Saginaw River/Bay Area of Concern are defined as extending from the head of the Saginaw River, at the confluence of the Shiawassee and Tittabawassee rivers upstream of Saginaw, to its mouth, and all of Saginaw Bay out to its interface with open Lake Huron at the imaginary line drawn between Au Sable Point and Point Aux Barques. Areas outside these physical boundaries, but within the Saginaw Bay basin, are considered in the RAP if they are sources of contaminant materials delivered to the Saginaw River and/or Saginaw Bay.

Environmental Concerns

Saginaw Bay is an important and unique ecological, economic and recreational resource to the state of Michigan. Water drawn from the bay is used as a source of drinking water for over 300,000 people, and for industrial water supply to an extensive industrial infrastructure. The shallow, nutrient-rich waters support extensive coastal wetland areas, which provide important spawning, nursery and feeding areas for many of the over 90 species of fish reported from Saginaw Bay. The wetlands also provide important habitat to many waterfowl as the bay is located on a major migratory flyway. The bay is used for extensive recreational boating and commercial navigation. Sport and commercial fishing are important activities with sport fishing taking place year-round, drawing anglers from other states and throughout Michigan. Saginaw Bay sport fishing generally accounts for over 60% of the total Lake Huron catch in Michigan waters. The Saginaw Bay shoreline provides important recreational opportunities for swimming, picnicking, hiking and bird watching. Finally, the bay is important for its aesthetic qualities.

Unfortunately, past waste disposal practices and poor land use activities have degraded water quality of the Saginaw River and Saginaw Bay. Anthropogenic inputs to Saginaw Bay have been dominated by agriculture, which is the most extensive single category of land use in the watershed, in the rural areas of the basin, and by industrial and municipal wastewater discharges from urban areas.

Industry is quite diversified in the Saginaw Bay basin due to a wide range of natural resources, a well developed transportation network, and the early establishment of automobile manufacturing and related primary industries. The transportation equipment industry remains the largest employer in the basin and is located almost entirely within the Saginaw River watershed cities of Bay City, Saginaw and Flint. Other large industries include fabricated and primary metals, nonelectric machinery, chemicals, electronic equipment, and food processing.

Three major water quality issues have been identified as causing degraded environmental conditions and impairing designated uses in the Saginaw River/Bay system and these are cultural eutrophication, bacterial contamination, and toxic material contamination. Excess nutrients in Saginaw Bay have created eutrophic conditions with nuisance population levels of blue-green algae which have caused taste and odor problems in public drinking water supplies at the point of water intake. Eutrophication in the Saginaw River has also contributed to low dissolved oxygen levels in the river. Combined sewer overflows in the city of Saginaw during wet weather events have resulted in elevated bacterial counts in the Saginaw River and the issuance of public health warnings on the river. Fish tissue contamination by PCB in Saginaw Bay fish, and by both PCB and dioxin (2,3,7,8-TCDD) in Saginaw River fish, has resulted in the issuance of public health fish consumption advisories.

The goal of the Saginaw River/Bay RAP is to restore all designated uses that are presently impaired because of degraded water quality conditions. This goal is expressed in terms of three specific objectives. The first is to reduce toxic material levels in fish tissue to the point where public health fish consumption advisories are no longer needed for any fish species in the AOC. Presently, there is an advisory warning against the consumption of carp and catfish in both the Saginaw River and Saginaw Bay. The advisory also suggests that people restrict their consumption of all game fish species in the Saginaw River, and limit consumption of lake, brown and rainbow trout in the bay. There are no advisories for Saginaw Bay on the two principal sport fish species, these being walleye and yellow perch.

The second objective is to reduce toxic material levels in ambient water throughout the AOC to those of Michigan's water quality standards. This is an ambitious and long-term objective for certain toxicants such as PCB. For instance, right now Michigan's PCB water quality standard is 20 ppq, which is not only below the current analytical level of detection, but is also below PCB levels presently measured in the open waters of the Great Lakes and in rain water. Ambient water PCB concentrations measured at the mouth of the Saginaw River in 1989 were approximately 17-18 ppt and remained elevated relative to upstream and bay water PCB concentrations that were on the order of 3-5 ppt.

The third objective is to reduce eutrophication in Saginaw Bay to a level where the bay will support a balanced mesotrophic biological community. Doing so should eliminate the nuisance levels of blue-green algae populations, which are the source of the taste and odor problems in drinking water supplies drawn from the bay. It may also allow the reestablishment of the *Hexagenia limbata* mayfly population, thereby providing important forage for bay fish populations.

Toxic Materials

Extensive efforts have been made to reduce the discharge of toxic materials to the Saginaw River/Bay AOC. For example, Michigan has made great progress in stopping the discharge of PCBs and has a goal of eliminating all point source discharges. Presently there are only three known remaining point source discharges of PCBs in the Saginaw Bay watershed and these contributed at a total of only 4.4 kg of PCBs during 1987. This is a relatively small amount -- less than 9% of the 53 kg/yr estimated as being contributed by atmospheric deposition in 1980, and less than 1% of the 458 kg/yr load calculated at the mouth of the Saginaw River in 1980.

Nevertheless, fish consumption advisories remain in effect for the Saginaw AOC. Additionally, recent studies suggest that toxic contaminants may be impacting the reproductive success of some piscivorous birds. Since ambient water concentrations of toxic materials are quite low, it is thought that sediments in the AOC, which were contaminated by historical discharges, may be acting as a source of toxic materials to the aquatic food chain. Sediments in both the Saginaw River and Saginaw Bay have elevated levels of PCBs, arsenic, cadmium, chromium, copper, lead, nickel and zinc. Additionally, Saginaw River sediments may have elevated levels of dioxins and furans.

Sediments in the Saginaw River are most contaminated in, and immediately downstream of, the two major urban centers of Saginaw and Bay City (Figure 3.5.2). The most heavily PCB contaminated area is just downstream of the Bay City WWTP. In 1980, surficial sediment PCB concentrations averaged about 10 ppm with a maximum of 23 ppm. The contamination covered the entire width of the river in contaminated areas including the dredged navigation channel. Contamination also extended somewhat upstream of source discharge points as a result of reverse flow conditions, which can occur in the low gradient (approximately 1 inch/mile) Saginaw River because of wind induced seiche conditions in Saginaw Bay. Reverse flows in the Saginaw River have been noted all the way up to its confluence with the Tittabawassee and Shiawassee rivers, 22 miles upstream.

The 1980 data also showed that the PCB contamination was even greater in deeper sediments. In a 25-30 cm deep section of a sediment core collected downstream of the Bay City WWTP, a PCB concentration of 574 ppm was measured (Figure 3.5.2).

In Saginaw Bay, there is a large sediment depositional zone in the inner bay north of the Saginaw River mouth. The surficial PCB concentrations in 1980 were generally in the 0.5-1.0 ppm range. However, this area is so large that the amount of PCB estimated to be in the active sediment layer in 1980 was 3.7 metric tons.

More recent sediment samples from the Saginaw River and Saginaw Bay were collected in 1988. Over 200 ponar grab samples and 22 sediment cores were collected to assess present conditions and the impact of a once-in-100 year flood, which occurred in the Saginaw River in 1986. The final laboratory analytical results were just reported in April 1990 and consequently, data interpretation has not yet been completed. However, initial data inspection indicates the average surficial PCB concentrations have decreased about one order of magnitude since 1980 in both the river and the bay.

The highest surficial PCB concentration observed in the Saginaw River was 4 ppm and the highest observation from a core slice was 18 ppm in a 30-40 cm deep section. In Saginaw Bay, the highest surficial PCB concentration was 0.5 ppm. Though PCB concentrations in deeper sediments of Saginaw Bay were typically greater than corresponding surficial concentrations, they never exceeded 0.8 ppm.

Several metals remain above the "heavily polluted" criteria under the U.S. EPA open lake dredge disposal guidelines. These metals and their maximum 1988 surficial values (ppm) in the Saginaw River are copper (76), lead (90) and zinc (540). As was the case with PCBs, metal concentrations were higher in deeper sediments and maximum values in core slices were cadmium (6), chromium (221), copper (174), lead (144), nickel (79) and zinc (898).

Sediment samples collected from the Saginaw River navigation channel by the U.S. Army Corps of Engineers (ACOE) in 1988 indicated for the first time that there may be elevated levels of dioxin in Saginaw River sediments. A previous 1983 survey had detected no dioxin in eight samples analyzed at detection limits of 10-20 ppt. However, in 1988, duplicate analyses of a sample at a site near the Zilwaukee Bridge (Interstate 75), just downstream of Saginaw, obtained 2,3,7,8-TCDD concentrations of 110 ppt and 290 ppt. Two of the other nine samples also had measured concentrations of 2,3,7,8-TCDD of near 100 ppt. Maximum concentrations of 2,3,7,8-TCDF were about an order of magnitude higher at 1200-1500 ppt.

Recent sediment sampling in December 1989 and spring 1990, has been conducted in the lower eight miles of the Saginaw River as part of the U.S. Environmental Protection Agency's Assessment and Remediation of Contaminated Sediments program. Data are not yet available from this effort, but it has been reported that many of the cores inspected visually have alternating strata of black oily material and clay or sand. In general, the substrate of the Saginaw River is sand, with depositional areas of fine clay and silt.

Because of the contamination of sediments in the navigation channel, sediments that are dredged from this area are placed in a confined disposal facility (CDF) in Saginaw Bay approximately one mile from the mouth of the Saginaw River. There has been concern that PCBs may be leaking out of the CDF and contaminating the environment. In 1987 and 1988, the U.S. EPA and U.S. ACOE conducted studies to measure any leakage. Results of this project indicated that PCB leakage was negligible.

Eutrophication

Excessive phosphorus inputs to Saginaw Bay have impacted biological communities by creating eutrophic conditions that favor nuisance species and inhibit more desirable species. Extensive blue-green algae blooms created taste and odor problems in drinking water supplies drawn from the bay as recently as the late 1970s. Of the four drinking water intakes on Saginaw Bay, the Saginaw-Midland water intake, at Whitestone Point on the northwest side of the bay, accounts for about 85% of the water drawn from the bay for potable use. In 1974, this intake had taste or odor problems on 56 days, but by 1980 this had decreased to zero and there have been no reports of taste and odor problems at this facility since then. However, the Bay City drinking water intake, located in southern Saginaw Bay near the Saginaw River mouth, still has occasional taste or odor problems during the summer months.

The decrease in taste and odor problems at the Saginaw-Midland water intake during the 1970s indicated that the bay was becoming less eutrophic. Indeed, when the Saginaw Bay phytoplankton community was last surveyed in 1980, blue-green algae population levels had decreased substantially from those of the mid-70s. There was also a favorable shift in the phytoplankton community with the almost complete disappearance of the nuisance blue-green algae, *Aphanizomenon* and *Anacystis*. However, there remained a couple of problem areas related to phosphorus sources and bay circulation.

The flow of water into, around, and out of Saginaw Bay varies with wind direction and intensity. The most common circulation pattern is for flow to move south along the west side, around the south end, and north along the east side. There is a shallow shoal area extending across Saginaw Bay, approximately midway along its length, between Charity Island and Sand Point. This shoal area, combined with constriction of the shoreline in this area, tends to divide the Saginaw Bay water mass into two areas – an inner bay and an outer bay. As a result, there is a secondary, counterclockwise gyre around the inner bay. Consequently, the Saginaw River discharge tends to move east and north along the east side with some flow circulating back around the inner bay. As a result of these flow patterns, areas that still had populations of nuisance blue-green algae were the Sebewaing/Wildfowl Bay area on the eastern shore, and along the eastern shoreline north of Wildfowl Bay.

In addition to blue-green algae populations, other components of the phytoplankton and zooplankton communities showed decreases in the population sizes of eutrophic organisms in 1980. These community changes appear to have been the result of decreases in phosphorus loads to Saginaw Bay, though phosphorus concentrations in bay water remain higher than anywhere else in Lake Huron and, when last surveyed, the benthic macroinvertebrate community was composed primarily of pollution tolerant forms such as the aquatic worms *Limnodrilus* and midges *Chironomus*.

Total phosphorus concentrations in Saginaw River water decreased 40% from 1974 to 1980, and dropped another 25% from 1980 to 1986. Orthophosphorus concentrations (the bioavailable fraction) declined even more dramatically, falling 70% by 1980 compared to the mid-70s. This has resulted in declining phosphorus levels in Saginaw Bay, though not of as great a magnitude as in the Saginaw River. It is thought that phosphorus concentrations in Saginaw Bay have not fallen proportionally because of the periodic resuspension of bay sediments, and the associated sediment bound phosphorus, from wind driven resuspension events.

The phosphorus concentration reductions in the Saginaw River have been brought about by several actions including the 1977 state ban on the use of high phosphate detergents, reductions in phosphorus discharges from industrial and municipal wastewater treatment plants due to facility upgrades and better operation, and the implementation of various best management practices by area agricultural producers. This resulted in a 79% decrease in phosphorus loads to Saginaw Bay from these sources between 1974 and 1986, decreasing from 800 tonnes to 169 tonnes. Additional reductions in phosphorus loads to the bay are needed, however, to further reduce eutrophic conditions. Studies during the early 1980s indicated that roughly 55% of bay phosphorus loads came from fertilizer runoff from cropland, while 17% originated from other nonpoint sources. This supports the present phosphorus reduction strategy that includes major nonpoint source control efforts.

Conclusion

The Saginaw River/Bay Remedial Action Plan describes a variety of actions that are needed to further address the water quality problems just discussed. The cost of implementing the 101 actions identified in the RAP is estimated to be $170 million over the next ten years. This estimate does not include any costs for sediment removal or treatment if needed.

The ARCS program is providing important information on the areal extent of sediment problem areas, the toxicity of sediments, and contaminant bioaccumulation potential. The remediation techniques being investigated under the ARCS program are of great interest to Saginaw RAP participants. The potential for bioremediation is of particular interest because of the large areal extent of the sediment contamination problem, particularly in Saginaw Bay, and the encouraging recent findings with respect to biological degradation of PCBs in sediments.

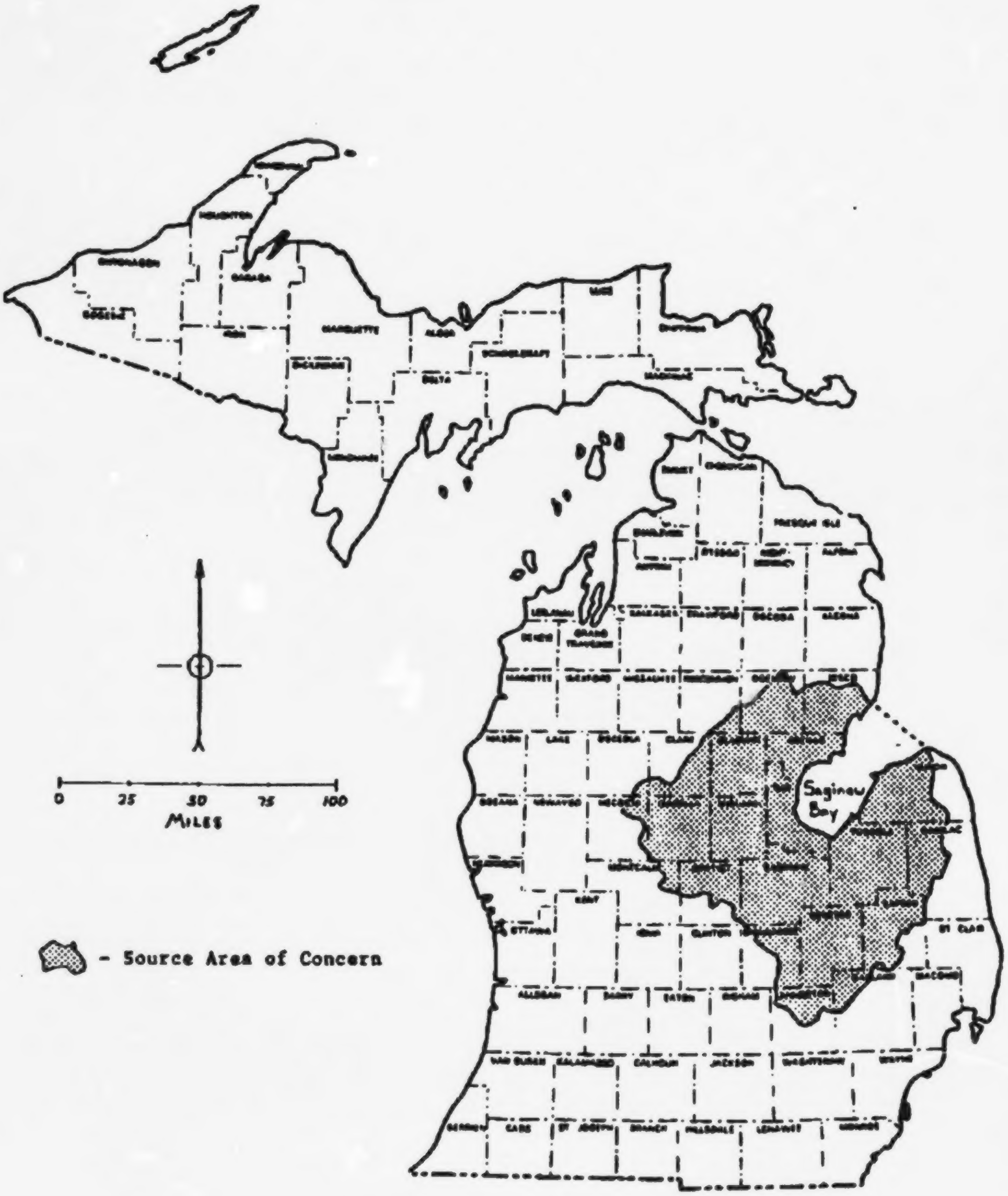
Saginaw Bay
0
25
50
75
100
MILES
- Source Area of Concern
ONTONAGON
MARQUETTE
SCHOOLCRAFT
GRAND TRAVERSE
OSCODA
ALCONA
MASON
LAKE
OSCEOLA
CLARE
OCEANA
NEWAYGO
MECOSTA
KENT
OTTAWA
IONIA
CLINTON
ST CLAIR
OAKLAND
MACOMB
ALLEGAN
BARRY
EATON
INGHAM
LIVINGSTON
VAN BUREN
KALAMAZOO
CALHOUN
JACKSON
WASHTENAW
WAYNE
BERRIEN
CASS
ST JOSEPH
BRANCH
HILLSDALE
LENAWEE
MONROE

Figure 3.5.1. Location of the Saginaw River/Bay Area of Concern

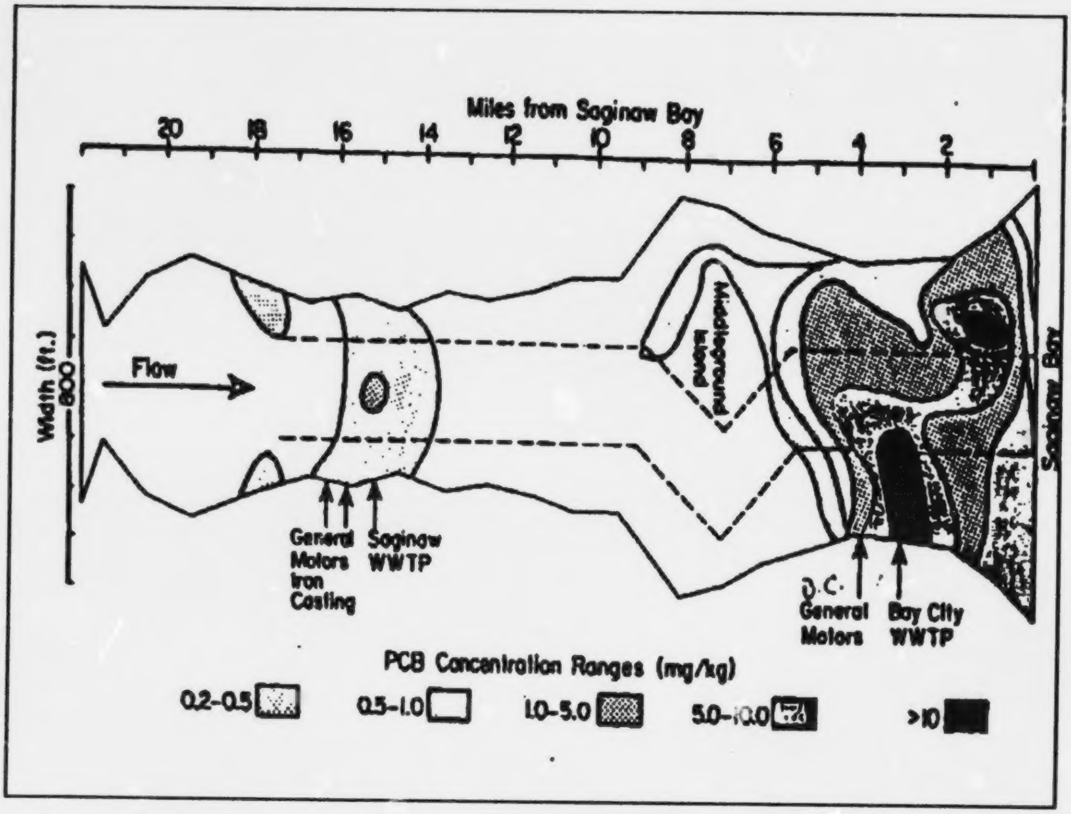
Miles from Saginaw Bay
20
18
16
14
12
10
8
6
4
2
Width (ft.)
800
Flow
Middleground Island
Saginaw Bay
General Motors Iron Casting
Saginaw WWTP
D.C. General Motors
Bay City WWTP
PCB Concentration Ranges (mg/kg)
0.2-0.5
0.5-1.0
1.0-5.0
5.0-10.0
>10

Figure 3.5.2. Spatial distribution of PCBs in surficial sediments of the Saginaw River

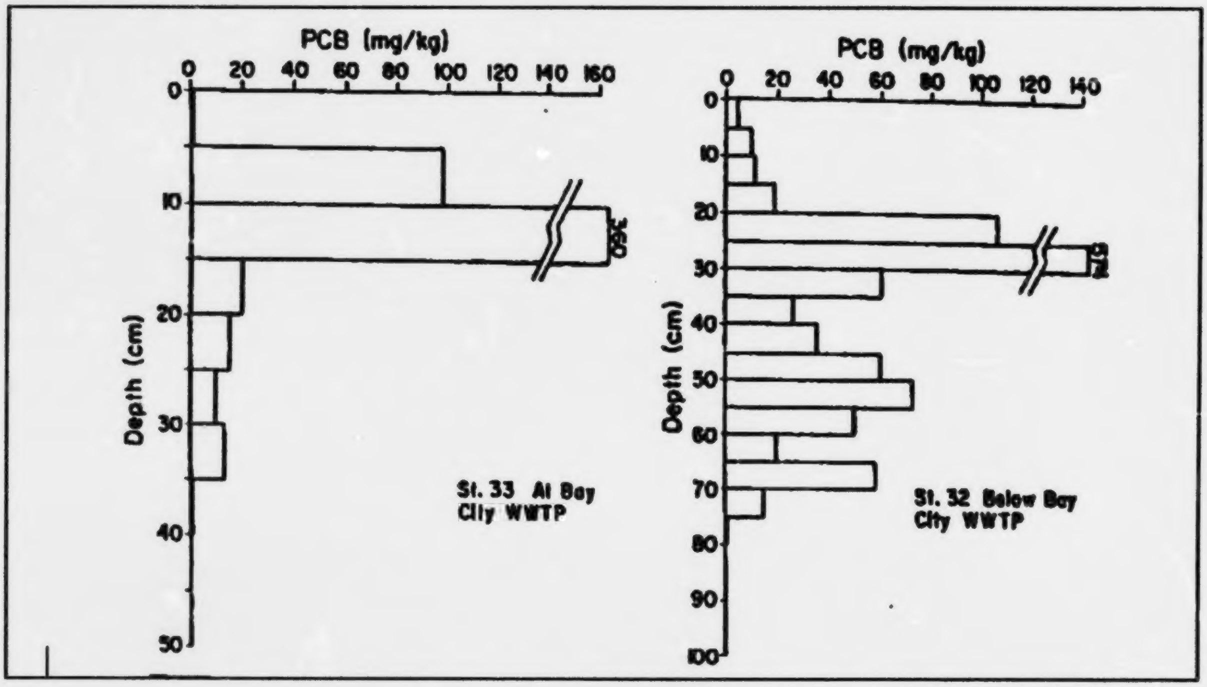

Figure 3.5.3. Vertical distribution of PCBs in sediments near Bay City WWTP

3.6 Sheboygan River and Harbor, Sheboygan, Wisconsin

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I. Site Background/History

The SRH site is located near and on the western shore of Lake Michigan, approximately 55 miles north of Milwaukee in the State of Wisconsin. The site includes approximately 14 miles of the Sheboygan River, and the Sheboygan Harbor, which is about 96 acres in size. In the 1950's, the ACOE began dredging the lower Sheboygan River and Harbor annually, depositing the sediments in offshore waters of Lake Michigan. Dredging was discontinued in 1969 when sampling of Harbor sediments revealed them to be contaminated with heavy metals. Due to routine fish sampling undertaking by the WDNR in which fish were found to have elevated concentrations of PCBs, U.S. EPA sampled sediments from both the River and Harbor in 1977 and found them to be contaminated with PCBs in concentrations exceeding 50 ppm. As a result, ACOE plans for a CDF and any further dredging were put on hold due to concerns for impacting public health and lack of an upland disposal site.

The SRH site was evaluated under the U.S. EPA Hazard Ranking System (HRS) due to the PCB and heavy metal contamination of sediments and the PCB contamination of the fish. Based on its HRS score, the SRH site was nominated for inclusion on the final NPL in May 1986, U.S. EPA and Wisconsin Department of Natural Resources signed a Consent Order with Tecumseh Products Company, one of the three PRPs identified, requiring the Sheboygan Falls-based company to conduct a Remedial Investigation/Feasibility Study (RI/FS). The contractor for Tecumseh, Blasland & Bouck Engineers (B&B), began the RI/FS in the Spring of 1986.

II. RI/FS

The objectives of the RI/FS were to determine:

1. the hydraulic characteristics of the river;
2. sediment characteristics and horizontal and vertical distribution of contaminants;
3. sediment mobilization, diffusion and transport phenomena;
4. the affinity of the PCBs and other contaminants for various sediment particle sizes;
5. the level of contamination in the water column.

The RI/FS incorporated a unique approach, for that time. Called a "Phased Approach" to conduct an RI/FS, the RI incorporated certain FS tasks early on by collecting only the amount of information sufficient for making decisions concerning the development and screening of potential remedial alternatives. This would allow for additional investigative efforts to be performed during an Alternative Specific Remedial Investigation Feasibility Study phase. These could include pilot studies, bench-scale studies, treatability studies, congener-specific PCB analysis, biodegradation assessment, etc.

III. RI/ES Results

A draft Remedial Investigation/Enhanced Screening (RI/ES) Report was completed in 1988 and revealed that site sediments are highly contaminated with PCBs and a variety of toxic metals including chromium, cadmium, lead, mercury, zinc, and nickel. The upper 2 3/4 mile stretch of the river was determined to be the major source of PCBs to the site, with elevated PCB concentrations found to be as high as 4500 ppm. Two dams in the Village of Kohler restrict water flow, thereby causing sediments to drop out. As a result, the contaminated sediments tend to be confined to this upper segment of the river. The next segment of the river, from the second Kohler dam to the Pennsylvania Avenue bridge in Sheboygan, conversely found PCBs to range from non-detect (ND) to less than 20 ppm. For the lower river and harbor, the lower river (inner harbor) found the next greatest levels of contamination with PCBs ranging from 0.03 to 0.220 ppm with all concentrations within the top 2 feet less than 21 ppm. The remainder of the harbor, the outer harbor, found PCBs ranging from ND to 1.1 ppm. Note: ND is 0.025 ppm. As to water column, the highest measured total (unfiltered) PCB concentration was 0.27 ppb under moderate flow conditions (about 200 cfs). Other flow regimes (low and low-moderate found maximum concentrations of PCBs tended to follow the pattern of river sediments with the highs of 71 ppm and 30 ppm in the uppermost segment of the river.

The Agency's review of the Endangerment Assessment concluded that there exist two exposure scenarios posing unacceptable risks to human health (i.e. the calculated cancer risk level exceeds 10 E4). These two scenarios are:

1. dermal exposure to river sediments;
2. ingestion of several species of fish and waterfowl.

The Sheboygan River can be easily accessed and is used by the public for a variety of activities including canoeing, fishing, wading, and hiking along the shoreline. Fish consumption advisories to not eat fish from the river and harbor have been issued by the WDNR for over 11 years, while consumption advisories against eating waterfowl caught in the area have been issued for the past 3 years.

The enhanced Screening (ES) segment identified potential remedial technologies and constructed potential remedial alternatives. These alternatives were then evaluated and screened based on effectiveness in reducing the contaminant toxicity, mobility, or volume; technical feasibility; and administrative feasibility, including potential public acceptance. The ES segment concluded with a listing of the remaining alternatives including *in situ* remedial alternatives; sediment removal, treatment, and disposal alternatives; and the no action alternative. The ASRI grew out of the RI/ES, specifically to address questions regarding the feasibility of many of the innovative technologies identified as part of these remedial alternatives.

IV. ASRI

The purpose of the Alternative Specific Remedial Investigation is to study and evaluate innovative technologies which may be used in remediating PCB-contaminated sediments found in the river. The goal of this study is to generate information to help determine an appropriate course of remedial action at the SRH site. A side benefit to be realized from this study is the minimization of human health and environmental risk due to the removal of the most highly contaminated sediments from the river.

The ASRI is a multi-faceted study including:

1. A Pilot Confined Treatment Facility (CTF) to study enhanced natural biodegradation for treatment of PCB-contaminated sediments removed from the Sheboygan River, and to test certain design components to evaluate their full-scale feasibility as a remedial alternative.
2. Evaluation of sediment removal technologies.
3. Evaluation of sediment control devices, i.e. silt curtains and other measures to prevent and control resuspension of sediments.
4. *In situ* armoring of low PCB concentration sediments.
5. A monitoring program designed to assess the effectiveness of the removal, armoring and biodegradation of sediments.
6. Bench-scale studies of removed sediments performed under laboratory conditions,

including PCB extraction, chemical fixation, armoring, supplemental biodegradation, dewatering and physical characterization.

Pilot Confined Treatment Facility

A Confined Treatment Facility (CTF), built on-site on property owned by Tecumseh, is being utilized to study the effectiveness of enhanced natural biodegradation for treatment of PCBs in sediments. Sediments with maximum PCB concentrations ranging from 640 ppm to 4500 ppm will be utilized in the pilot study. Additionally, certain physical components of the CTF are being studied to evaluate the feasibility of a full-scale CTF, including a series of permeable treatment walls (i.e. water treatment system).

Preliminary studies by Dr. John F. Brown, Jr., of General Electric's Research and Development Center, have shown that PCBs in the river and harbor sediments apparently are being transformed by at least three processes. According to his letter reporting his findings to B&B, which is in Appendix J of the RI/ES Report, Dr. Brown reports that "...PCBs in the river and harbor sediments are being transformed by two types of reductive dechlorination processes... and one type of oxidative biodegradation (process)."

In addition, bench scale biodegradation tests have been in progress at the University of Michigan. The results of these tests will be used to develop operating parameters for the CTF. Data will be forthcoming at their completion in the Fall.

The CTF has a capacity for approximately 1500 cubic yards of sediments, which are being dredged from the upper portion of the river. The CTF is divided into four treatment cells which will provide different testing environments in which to study the effectiveness of degrading PCBs by enhanced natural biodegradation.

Two of the treatment cells will receive enhancements, such as a nutrient mixture, a carbon source, or a surfactant. Other factors may also be controlled, including oxygen and pH. The types of enhancements, rate of application, and control of other factors will be determined through the on-going bench-scale biodegradation studies at the University of Michigan. Biodegradation will be studied under both anaerobic and aerobic conditions. It is expected that each of the treatment cells will undergo both anaerobic and aerobic degradation cycles. The two remaining cells will act as control cells where bacterial activity is not enhanced or controlled.

The CTF is constructed of structural steel sheet piling, 25 feet in length, driven 15 feet into the ground. Facility dimensions measure approximately 106 feet long by 135 feet wide by 10 feet high. The 14,000 square foot facility has a double liner in each of the cells and incorporates a leak detection/leachate removal system in-between the two liners. Overflow protection is also provided for through the use of piping to collect excess water to the treatment system. A piping system has been placed into each cell for aeration, drainage, or addition of enhancements. The water treatment system consists of four permeable treatment walls which will be used to evaluate four different mediums for filtering the water thereby removing the PCBs. A backup carbon adsorption system will also be utilized to ensure a discharge that meets effluent requirements.

Sediment Removal

Extensive sampling and analysis of river sediments have identified specific depositional areas for removal. Dredging has been accomplished through the use of mechanical equipment. A crane with a modified clamshell bucket was used, from either a land base or from a barge. The clamshell bucket was modified whereby the joints were sealed to minimize losses of dredged material to the water column. Operational controls were utilized to reduce resuspension - through controlling the speed of the bucket through the water column, maintaining a smooth movement of the bucket, and not dragging the bucket over the dredged bottom to smooth it out. In addition, sediment control devices consisting of double-lined siltation control curtains were placed completely around the sediment area prior to dredging to contain any resuspended sediments. Sediments were placed into a sealed box which was then transported by barge or truck to the CTF.

To ensure that all prescribed sediments were removed, removal was conducted in two steps.

In step one, the majority of the sediments were dredged to a depth based on study data. Suspended particles within the siltation control curtains were then allowed to settle at least 24 hours. The second-step dredging operation removed an additional six-inch layer comprising any settled solids and allowing for a safety factor. Following another period to allow any sediments to resettle, the area was probed for any remaining sediments and samples were collected for PCB analysis. Based on the probing and sampling results, either additional material was removed or the siltation control curtains were removed.

Sediment Armoring

Armoring confines the sediments in place by covering them with successive layers of materials in order to minimize their resuspension and to minimize or stop the loss of PCBs to the water column. This pilot study will also assess the effects of armoring on in-place biodegradation of PCBs.

Approximately 20,000 square feet of sediments will be armored in place. After placement of siltation control curtains around the sediment area to be armored, a geotextile material is first placed over the sediment area. A line of sandbags temporarily holds the fabric in position. The sediment area is then armored with roadbed material consisting of fine- to coarse-grained material. A second layer of geotextile is then placed over the roadbed material and gabions are placed around the edges to permanently hold the fabric in place. The sandbags were previously removed. A layer of cobbles is placed over the geotextile. Finally, a layer of roadbed material is spread over the gabions. Once any resuspended solids (from the roadbed material) had settled, the siltation control curtains were removed.

Monitoring

An extensive monitoring program has been established in order to evaluate the effectiveness of the activities associated with the removal, armoring and biodegradation of PCB-contaminated sediments. This program has incorporated sampling and analysis of the water column, sediments, and fish as follows:

1. Baseline sampling and analysis of the water column for PCBs (filtered and unfiltered), total suspended solids, volatile suspended solids, turbidity;
2. During removal and armoring activities, weekly water column sampling and analysis for PCBs, TSS;
3. Daily monitoring of the water column during removal and armoring activities for TSS and turbidity;
4. Sediment and water sampling for PCB analysis within silt curtained area post-dredging;
5. Long-term sampling of resident fish - PCBs and lipid content;
6. Caged fish studies using fathead minnows and tethered clams are being conducted pre-, during and post- removal/armoring activities - for PCBs and lipid content;
7. Sediment sampling for congener-specific PCBs to evaluate biodegradation in CTF and under armoring materials.

V. Initial Results

The analytical results for samples of the water column, sediments, native species of fish, fathead minnows, and clams have been coming in over the past several months, and will continue over the next 1 to 2 years. Once the data has been reviewed and compiled, it will be released. The analytical results thus far indicate that there has been no or minimal measured impact on the water column due to sediment dredging and armoring activities, based on analysis for TSS, turbidity, and PCBs. Preliminary conclusions show that the use of double-lined siltation control curtains and operational controls by the crane operator are successful in controlling and preventing the loss of resuspended sediments and PCBs into the surrounding water column during these activities. Samples collected after the completion of two rounds of dredging have shown that mechanical dredging using a modified clamshell bucket has reduced PCB concentrations as follows: 4500 ppm to 4.9 ppm; 830 ppm to 2.5 ppm; and 1000 ppm to

0.49 ppm.

VI. Future ASRI Activities/Project Schedule

Future activities include completion of the ASRI Pilot Study and the Feasibility Study. The following lays out the current draft schedule of activities:

ASRI Activities

- Complete dredging of sediment - July 1990
- Complete armoring of sediments - Fall 1990
- Continued monitoring activities - On-going
- Finalization of operational parameters from bench-scale biodegradation studies for enhancement of PCB biodegradation in CTF pilot Study - Sept. 1990
- Evaluation/monitoring of pilot CTF & armoring - Through Oct. 1991
- ASRI Report - Late 1991

FS Activities

- ARARS Finalization - Sept. - Dec. 1990
- Determination of clean-up standards
- Draft FS Report- March 1992
- Record of Decision - 4th quarter 1992

References

1. Sheboygan River and Harbor Remedial Investigation/Enhanced Screening Report, May 1990. Prepared by Blasland & Bouck Engineers.

2. Sheboygan River and Harbor Alternative-Specific Remedial Investigation Work Plan/QAPP, August 1989. Prepared by Blasland & Bouck Engineers.

3. Sheboygan River and Harbor Superfund Site File. U.S. EPA Region V.

4 POLYCHLORINATED BIPHENYLS (PCBs)

4.1 Aerobic Biodegradation of PCBs

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On the spectrum of chemical targets from easiest to most difficult, the polychlorinated biphenyls are definitely one of the more challenging for bioremediation. Research programs over the last 18 years have clearly demonstrated that PCBs can be biologically destroyed by bacteria and fungi. However a continuing development effort will be required to transition some very promising laboratory results to commercial cleanup technologies.

Unlike easier targets such as gasoline and simple pesticides, the microbes which degrade PCBs do not utilize them as a source of carbon and energy. These bacteria break down PCBs in a cometabolic process whereby the organism grows on one substrate, for example, biphenyl, and then fortuitously degrades the target substrate, in this case PCBs. There is no energy derived from the breakdown of PCBs and in some cases this process consumes energy. Therefore, whereas indigenous microbes are generally sufficient to degrade simple targets because of their selective growth advantage, aerobic technologies to degrade PCBs will probably require the introduction of exogenous organisms. Another challenge posed by PCBs is their insolubility with the result that some PCBs are often not bioavailable. Therefore, some of the technology efforts currently underway are attempting to develop physical and chemical pretreatment and cotreatment approaches for increasing the bioavailability of this difficult substrate.

Generally, one can consider two approaches for a PCB bioremediation system. The first would be an *in situ* approach whereby bacteria would be introduced directly to the contaminated soils or sediments and then incubated under conditions to facilitate the degradation of the target. Alternatively, one can excavate the soils or sediments and treat them in a soil slurry bioreactor with added microbes and nutrients. For strictly aerobic biodegradation of PCBs, the latter is the most promising technology for the near term. However, *in situ* approaches are a major goal of this technology and in the short term may be most applicable for anaerobic sediments.

Discoveries over the last several years have now shown that PCBs can be broken down by both aerobic and anaerobic microbial systems. This paper will discuss solely aerobic approaches for the biodegradation of PCBs. However, other papers at this meeting will address the complementary anaerobic technologies.

It is important to keep in mind that PCBs are a complex family and not a single chemical target. There are 209 different theoretical PCB congeners from mono- through decachlorobiphenyl. However, all do not exist in the environment. Generally, the more chlorine atoms on the molecule, the more recalcitrant it is. However, the position of the chlorine atoms is also a critical factor in the biodegradability of PCBs. For example, 2,5,2',5'-tetrachlorobiphenyl is readily degradable by Type II bacterial strains, whereas 3,5,3',5'-tetrachlorobiphenyl and 2,6,2',6'-tetrachlorobiphenyl are not biodegradable to any extent by any known bacterial species.

As produced by Monsanto under the trade name Aroclor, each PCB mixture contained from 30-60 congeners. Aroclors 1242 and 1248 are more easily biodegradable by aerobic systems than the higher chlorinated mixtures. These two Aroclors contain mostly di-, tri-, tetra, and some pentachlorobiphenyl. The congeners in Aroclors 1254 and 1260 contain tetra-, penta-, hexa-, and heptachlorobiphenyls and pose a much greater challenge to aerobic bacteria. In the short-term, we believe that a strictly aerobic approach to PCB biodegradation will be limited to the lower Aroclors (1242 and 1248) and probably at concentrations no higher than 1,000 - 5,000 ppm. For the higher Aroclors (1254 and 1260) and for higher concentrations of the lower Aroclors, a dual anaerobic/aerobic biotreatment system will probably be required.

The initial studies to degrade PCBs from university and industrial labs throughout the world were all aerobic and the approach taken was generally similar. Soil from PCB contaminated sites was used to inoculate minimal media containing either biphenyl or monochlorobiphenyls as sole source of carbon and energy. From these enrichments, many bacterial strains have now been isolated which can degrade PCBs. These strains, however vary dramatically in their capabilities to degrade PCBs. Some can only degrade lower chlorinated congeners such as mono-, di-, and trichlorobiphenyls, whereas some cultures can degrade PCB congeners as highly chlorinated as tetra-, penta- and hexachlorobiphenyl. It is the use of these more active strains that will be the basis for commercial PCB bioremediation technologies.

In addition to the differing capabilities of PCB-degrading bacterial strains in terms of the chlorine content of the ring, another discovery has shown that at least two different types of bacteria exist which degrade complementary PCB congeners. This can be illustrated by two of Envirogen's more active strains, one of which (Type I) readily degrades PCB congeners which are substituted in both *para* positions (4,4'-chlorobiphenyl family), whereas the strain (Type II) has the capability of readily degrading PCB congeners with a 2,5-substitution pattern on one ring. The congener complementary of these two strains forms the basis for the current development program for a commercial aerobic PCB biotreatment system.

Biochemical studies of these and other strains have now elucidated the biodegradative pathways for PCBs. This pathway is similar to other aromatic compounds whereby the initial attack is by a dioxygenase followed by a dehydrogenase and subsequent ring cleavage by another dioxygenase. The end product from this initial oxidation is generally the chlorinated benzoic acids. Other microbes in mixed cultures have the capability of further breaking down chlorobenzoic acids to carbon dioxide and water. The molecular genetics of PCB-degrading strains is now under investigation and the genes from various of these organisms have been isolated from several laboratories, including Envirogen. It is the goal of these studies to develop superior PCB-degrading strains which will express higher levels of PCB-degradative enzymes. Additionally, the use of genetic engineering will permit us to uncouple biphenyl metabolism in these strains from PCB biodegradation, thereby allowing these cultures to be grown on a common inexpensive carbon source and then utilized as biocatalysts to degrade the target PCBs.

The initial microbiological, biochemical and genetic studies of PCB-degrading strains have now led to research and development projects with soils and sediments contaminated with PCBs. This, of course, is the ultimate goal of development programs and what is needed for addressing problems such as those in the Great Lakes Basin. Several laboratories are attempting to develop commercial systems for the biotreatment of PCBs on soils and sediments, however, it has been critical for biodegradation process-modeling research to demonstrate that bacterial soil decontamination results are unequivocally due to biological activity. One pitfall that both scientists and regulators must be concerned with is congener depletion in a "biodegradation" process that is actually due to physical loss of the PCB and not to true biological degradation. With Aroclor studies, these processes can easily be distinguished, because biodegradation results in depletion of specific congeners yielding gas chromatographic (GC) profiles that are distinctly different from those of the original Aroclor mixtures. Physical depletion, on the other hand, results in uniform depletion of all congeners (e.g., adsorptive loss) or depletion of lower-chlorinated congeners due to their higher volatility (e.g., evaporative loss). The production of PCB metabolites is of course another unequivocal method for demonstrating the biological basis of PCB depletion.

In order to better evaluate results for open-air, aerated, stirring reactors, a model process was set up to mock a biologically mediated treatment, but whose conditions were adjusted so as to preclude biodegradation (study conducted at GE, CRD, Schenectady, NY). A sample of soil contaminated with Aroclor 1260 (7800 ppm) was used in this study. Argon (instead of air) was bubbled through a water/soil slurry in a round-bottom flask. A florosil sample tube was attached to trap PCBs in the argon off-gas as it exited the vessel. Samples were taken periodically while mixing to ensure a homogeneous sample. The volume in the vessel was maintained by adding distilled water after each sampling, and the florosil sample tube was replaced each time a sample was taken. The soil was mixed and purged with argon at room temperature for 19 days, after which the vessel was disassembled.

Each soil sample and florosil tube was extracted for GC analysis. The remaining soil and water were removed and pooled for GC analysis. The entire vessel was extracted several times to remove any PCBs bound to the vessel. These extracts were also pooled for GC analysis. Upon disassembly, a tar-like substance was observed sticking to the Teflon stirrer. This was removed and added to the soil and water fraction for PCB analysis.

Neither oxygen nor bacterial inoculum was introduced in this mock process, yet the analytical results could be mistaken for biodegradation. Although the time-course analysis indicated greater than 90% PCB depletion, it was clear from the mass balance calculations (86% PCB recovered) that the aeration and stirring of the soil resulted in the redistribution of PCBs from soil to the difficult-to-sample locations in the reactor (i.e., glassware, stirrer, and coalesced droplets of PCB). The GC profiles also demonstrated that the observed depletion was not due to a biological process, since all GC peaks were depleted proportionally. Therefore, experiments that purport to show biodegradation of PCBs by quantifying total GC peak areas must be carefully evaluated. It is for this reason that nonbiodegradable PCB internal standards should be included wherever possible. If such standards or dead-cell controls cannot be included, then one must rely on differential congener depletion (or metabolite production) as evidence for the biological basis of PCB "biodegradation" processes.

Our studies to date are concentrating on utilizing Envirogen's two best Type I and Type II PCB-degrading strains to develop a strictly aerobic biotreatment process. Similar studies done at General Electric CR&D, both in the laboratory and eventually in a direct field application using one Type II microbe, clearly demonstrated that PCBs on soil can be biodegraded, however, several limiting factors were identified. In the General Electric experiments, a PCB-contaminated soil containing 500 ppm of an Aroclor 1248-like mixture could be degraded to the extent of 50 percent PCB destruction in an actively-mixed system in 1-3 days. In a laboratory modeled *in situ* approach, this extent of degradation was not achieved until 100 days of incubation. In field studies with the single Type II strain in an *in situ* mode, only 25 percent destruction was achieved in approximately 100 days (i.e., one-half that observed in the laboratory).

From those General Electric studies, several key parameters were identified as necessary in order to improve the extent of aerobic degradation from 50 to 90 or 99 percent. These parameters include bioavailability, temperature, and better utilization of microbial strains.

The issue of bioavailability is critical when developing treatment systems for highly insoluble substrates such as PCBs. Studies are currently underway to develop physical and chemical pretreatment approaches to facilitate the desorption of PCBs form soils and sediments, thereby increasing the rate of uptake by bacterial strains. In the short term, we believe that reactor-based technologies will show more promising results due to the active mixing in a soil slurry system as opposed to the diffusion limitations of *in situ* approaches. The bioavailability of PCBs can also be affected by co-contaminating substrates such as simple hydrocarbon oils. Studies at Envirogen have shown that a co-contamination oil at a PCB-contaminated site dramatically reduces the extent of PCB biodegradation by otherwise competent microorganisms. From this study, and others done at General Electric, it is apparent that PCBs are sequestered into the oil phase and are therefore not available for the PCB-degrading bacteria.

In terms of the best utilization of microbial strains, we believe that the use of co-cultures of Type I and Type II bacteria will result in the most extensive degradation of the broadest range of congeners. As discussed above, Type I strains preferentially degrade double-*para* substituted congeners and Type II strains are better able to degrade congeners with 2,5-substituted rings. Studies utilizing individual versus co-cultures of Type I and Type II strains have now clearly shown the advantages of using the two complementary strains in concert. For example, Envirogen strain ENV 307 can degrade 59 percent of Aroclor 1248 in a standard

30 ppm, 20-hour assay. Envirogen strain ENV 360 can degrade 58 percent. However, when both strains are utilized together, greater that 70 percent of the PCB is destroyed in these 20-hour assays.

Soil from a PCB-contaminated location which contains 290 ppm Aroclor 1248 has now been shown to be biodegradable down to levels of less than 100 ppm utilizing the two complementary strains. The challenge now is to develop this technology to get greater levels of destruction. One approach that one can envision is the development of a genetically-engineered strain which will encode both Type I and Type II PCB degradative pathways in a single organism.

Ultimately, the development of dual anaerobic/aerobic biotreatment systems will also allow us to address higher concentrations and higher chlorinated congeners by initially performing an anaerobic biotreatment step to first remove chlorine atoms from the biphenyl nucleus, thereby transforming Aroclors to lower chlorinated mono-, di-, and trichlorobiphenyl products. These are readily degradable by aerobic bacteria.

In summary, a direct aerobic biodegradation treatment technology is the first, short-term goal for biotreatment of PCB-contaminated soils and sediments. This will be reactor-based and address Aroclor 1242 and 1248 problems at concentrations under 5,000 ppm. Technology advances using anaerobic cultures, genetically-engineered strains, and soil pretreatment steps will in the future extend the capability of PCB biotreatment systems to higher Aroclors and higher PCB concentrations.

4.2 Anaerobic Dechlorination and the Bioremediation of PCBs

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Anaerobic reductive dechlorination is a newly recognized environmental fate of polychlorinated biphenyls (PCBs) that is of potential importance in risk assessment, deciding remediation strategies for contaminated sites, and in developing treatment systems for the biodegradation of the more heavily chlorinated PCB mixtures (Aroclors). Dechlorination both reduces the toxicity of a PCB mixture and makes it more aerobically degradable. Thus a sequential anaerobic/aerobic treatment system has the potential to degrade the more heavily chlorinated PCB congeners that are resistant to aerobic degradation.

We here review our research findings on PCB dechlorination and further discuss the implications for bioremediation of PCB contaminated sediment and soil.

First Evidence for Anaerobic PCB Dechlorination

The PCB congener distribution patterns obtained for core samples from the upper Hudson River first suggested the anaerobic dechlorination of PCBs (*2*). Aroclor 1242 was known to be the primary input to this section of the river, and surface sediments showed a congener profile similar to Aroclor 1242. Deeper and potentially anaerobic sediments, however, showed a depletion of the more chlorinated congeners and a corresponding increase in mono- and dichlorobiphenyls. This suggested that anaerobic bacteria might be dechlorinating the PCBs in the deeper sediments. Microbially mediated reductive dechlorination of other chlorinated aromatics had at that time been recently demonstrated (*13*). Similar differences between congener profiles for sediments and their most probable PCB inputs have since been observed at other sites (*1,3*).

Demonstration of Biologically Mediated PCB Dechlorination

We first demonstrated biologically mediated reductive dechlorination of PCBs by adding low concentrations of pure PCB isomers to PCB-contaminated Hudson River sediments. Dechlorination was observed, but attempts to obtain dechlorination in the absence of sediments were not successful. The high levels of putative dechlorination products in these sediments made it impractical to study the dechlorination of Aroclors by adding them to the contaminated sediments.

Methods

To study the dechlorination of Aroclors, we therefore developed a method in which microorganisms from the contaminated sediments were transferred to non-PCB contaminated sediments (*9,10*). First serum bottles or Balch tubes of methanogenic "clean" sediments were prepared and autoclaved. These were then inoculated with supernatant from a anaerobic slurry of a contaminated sediment. Control bottles or tubes were then autoclaved. An Aroclor was then added in a small quantity of acetone while flushing with filter sterilized nitrogen:carbon dioxide (80:20) and the vessels were sealed with Teflon lined rubber stoppers. Periodically samples were extracted and analyzed for changes in the PCB congener profile by capillary gas chromatography with an electron capture detector.

Dechlorination of Aroclor 1242 by Hudson River Microorganisms

Dechlorination of 700 ppm Aroclor 1242 (on a sediment dry weight basis) by microorganisms eluted from the Hudson River sediments was readily apparent from a visual inspection of the chromatograms for live samples taken after 16 weeks of incubation (Figure 4.2.1). There was a marked decrease in the peak heights for later eluting, more heavily chlorinated congeners and an increase in the early eluting mono and dichlorobiphenyls. Closer inspection revealed that nearly all of the dechlorination occurred from the *meta* and *para* positions (Figure 4.2.2). Little if any *ortho* dechlorination occurred. This resulted in the accumulation of mostly 2-chlorobiphenyl (2-CB), 2,2'-CB and/or 2,6-CB (coeluting isomers), and 2,2',6-CB. The detector response for 2-CB is particularly weak; while the peak height for this congener is small (bottom panel, Figure 4.2.1) this congener actually represented 63% of all PCBs recovered from the live samples receiving 700 ppm Aroclor 1242 after 16 weeks of incubation. Most of the other persistent congeners were chlorinated in both *ortho* and *meta* positions, indicating some preference for dechlorination of the *para* positions over the *meta* ones.

Concentration Dependence

The extent of dechlorination observed was concentration dependent (Figure 4.2.2), being greatest at 700 ppm. At that concentration the average number of *meta* and *para* chlorines decreased from 1.98 to 0.31 after 16 weeks, but decreased to only 1.19 in the 140 ppm treatment. There was no apparent dechlorination in the 14 ppm treatment.

There are two possible causes for the observed dependence on concentration. It may be related in part to bioavailability. Higher concentrations in the sediments should result in higher solution concentrations (*4*), and it may be that only PCBs in solution are available for microbial uptake (*8*). It is also possible that greater population growth of the dechlorinating organisms occurred during the experiment at the higher PCB concentrations resulting in more extensive dechlorination. Two subsequent experiments give credence to this interpretation. Dechlorination activity has been maintained through eight successive transfers in the presence of 1000 ppm Aroclor 1242. The activity would have been lost if no growth of the dechlorinating population occurred. In a second experiment, the dechlorination rates at 500 and 5000 ppm of Aroclor 1254 were compared. Initial dechlorination rates (calculated as Cl^- released per week) were similar for the first 8 weeks, but between 8 and 16 weeks the rate at 5000 ppm was 10 times higher than at 500 ppm.

Terminal Products

The high accumulation of the *ortho*-only substituted products in the above experiment suggested that they were terminal products. The high total recovery did not indicate that there was significant degradation of the biphenyl structure under our incubation conditions. We therefore conducted experiments in which only biphenyl, 2-CB, 2,2'-CB, or 2,6-CB was added to sediment slurries inoculated with Hudson River microorganisms. There was no evidence for the dechlorination or degradation of any of these compounds during a one year incubation. We therefore conclude that these are in fact terminal products under our experimental conditions.

Selection for PCB Dechlorinators

There appears to have been selection for PCB dechlorinating microorganisms at several PCB contaminated sites. Assays for the presence of PCB dechlorinating microorganisms in PCB-contaminated sediments generally give positive results within 4 weeks and extensive dechlorination within 12 to 20 weeks. In contrast, assays for the presence of these organisms in non-PCB contaminated sediments yield at most very modest activity after incubation periods of 20 weeks or more. We therefore believe that PCB-dechlorinating microorganisms may be widely distributed at low levels, but they are much more abundant at PCB-contaminated sites that are also otherwise favorable for their growth.

There may be two related advantages to microorganisms from the dechlorination of PCBs.

The dechlorination process may be serving as a terminal electron sink in anaerobic sediments. Terminal electron acceptors are usually the limiting factor for microbial growth in anaerobic habitats so that any microorganism that could use PCBs for this purpose would be at a selective advantage in such habitats. The second related advantage is that it is possible that energy can be gained from the dechlorination step itself. The chlorobenzoate dechlorinating strain DCB-1 can apparently gain energy from dechlorination (*5,6*).

Temperature Dependence

Dechlorination assays using Aroclor 1242 and Hudson River microorganisms were conducted at 12, 25, 37, 45, and 60°C. The greatest dechlorination rate was at 25°C while about half as much dechlorination occurred at 12°C (Figure 4.2.3). There was no dechlorination at temperatures of 37°C or above. Such temperature effects are characteristic of enzyme catalyzed reactions. It is also noteworthy that significant dechlorination can occur at normal environmental temperatures.

Dechlorination of Other Aroclors by Hudson River Microorganisms

The dechlorination of more heavily chlorinated Aroclors by the Hudson River microorganisms was also investigated (Figure 4.2.4). Aroclors 1242, 1248, 1254, and 1260 average approximately 3, 4, 5, and 6 chlorines per biphenyl, respectively. Dechlorination of all of these Aroclors was observed although the dechlorination rate and extent tended to decrease with increasing degree of chlorination, particularly for Aroclor 1260 (Table 4.2.1). As before there was no evidence for dechlorination from the *ortho* position. For Aroclors 1242, 1248, and 1254 only 2-CB and 2,2'-CB and/or 2,6-CB accumulated to an appreciable extent. The *meta* position was apparently more effectively dechlorinated than in our first Aroclor 1242 experiment. In the case of Aroclor 1260 the accumulation of *ortho* and *meta* substituted products, particularly 2,2',5,5'-CB, was noted.

From these and other experiments it appears that there are at least two distinct dechlorination activities associated with the Hudson River sediments. One preferentially dechlorinates the *meta* position while the other preferentially dechlorinates the *para* position. When both activities are expressed the only prominent products are the *ortho*-only substituted chlorobiphenyls.

Miscellaneous Observations

Several miscellaneous observations regarding PCB dechlorination by Hudson River microorganisms may be made from our attempts to characterize the dechlorinating microorganisms. While they can survive intermittent oxygen exposure, anaerobic conditions are required for dechlorination. Further, dechlorination has always been accompanied by methanogenesis. BESA (a specific inhibitor of methanogenesis), molybdate (an inhibitor of sulfate reduction), sulfate, and nitrate all inhibit dechlorination.

The frequency and amount of substrate added may influence PCB dechlorination. Continuously available yeast extract or acetate inhibited dechlorination. The dechlorinators may be out competed by other organisms when readily utilizable carbon sources are continuously available. Small amounts of pyruvate, fed at intervals such that all is consumed between additions, appear to support a dechlorinating community. Similarly made additions of glucose, methanol, or acetone appear to stimulate dechlorination (*7*).

Aroclor Dechlorination by Silver Lake Microorganisms

We have also investigated the dechlorination of Aroclors by Silver Lake microorganisms. Silver Lake in Massachusetts was contaminated primarily with Aroclor 1260 and some 1254. Initially the Silver Lake microorganisms dechlorinated Aroclor 1242 at a rate comparable to the Hudson River microorganisms (Table 4.2.1), but after the first 4 weeks dechlorination ceased, leaving an average of approximately one chlorine in the *meta* and/or *para* position. Closer inspection revealed the accumulation of *ortho* and *para* substituted products. Thus dechlorination of Aroclor 1242 by the Silver Lake microorganisms appears to be limited to removing primarily *meta* chlorines. Dechlorination ceased when most of the *meta* chlorines had been removed.

The Silver Lake microorganisms were more effective than the Hudson River ones at dechlorinating Aroclor 1260 (Table 4.2.1). Dechlorination was first evident after only 8 weeks of incubation and continued throughout the course of the experiment. As with Aroclor 1242, dechlorination appeared to be limited to removing *meta* chlorines. The most prominent dechlorination product was 2,2',4,4'-CB.

Dechlorination Patterns

The existence of the different dechlorination patterns or specificities for the Hudson River and Silver Lake microorganisms implies the existence of different PCB dechlorinating species or strains. We have found at least one other unique dechlorination activity associated with New Bedford Harbor, MA sediments.

Implications for Bioremediation

The anaerobic dechlorination of PCBs has three important implications for the development of a biological treatment system for the destruction of PCBs. First, because of the preferential removal of *meta* and/or *para* chlorines, anaerobic dechlorination alone reduces the toxicity of a PCB mixture. Second, the process is capable of transforming the more heavily chlorinated congeners that are so resistant to aerobic biodegradation. And third, if anaerobic dechlorination is coupled to a subsequent aerobic biodegradation step, then greater mineralization of the PCBs can be expected than from aerobic treatment alone.

Toxicity Reduction

The most toxic of the PCB congeners are generally considered to be the coplanar congeners 3,3',4,4'-CB, 3,3',4,4',5-CB, and 3,3',4,4',5,5'-CB. In a coplanar configuration, these congeners are structurally similar to 2,3,7,8-tetrachlorodibenzodioxin (TCDD) and exhibit similar toxicity effects. PCB congeners like these but with a single *ortho* chlorine also have similar toxicity effects but are much less potent.

We first confirmed the dechlorination of two of these toxic PCB congeners (3,3',4,4'-CB and 2,3,3',4,4'-CB) by adding them to the Aroclor 1242 used in a dechlorination assay with Hudson River microorganisms. These two congeners were dechlorinated at rates similar to other tetra and penta chlorobiphenyls in the Aroclor 1242 (Figure 4.2.5).

Both 3,3',4,4'-CB and 2,3,3',4,4'-CB coelute with other congeners in Aroclor 1242. Because we used an electron capture detector which does not distinguish among coeluting congeners, it was necessary to amend the Aroclor with each of these congeners in order to be sure they were being dechlorinated when present in a mixture. More recently Lopshire and Encke in the Department of Chemistry at Michigan State University have developed a sensitive GC-MS-MS technique to directly quantify these and other toxic PCB congeners. This new method allowed us to determine the percent reduction of each of the toxic congeners after a 16 week incubation of Aroclor 1242 with the Hudson River microorganisms.

The dioxin-like toxicity of compounds has been correlated with their potential to induce P_{450} enzymes such as aryl hydrocarbon hydroxylase (AHH) and ethoxy resorufin O-deethylase (EROD) and the toxicities of various PCB congeners have been estimated based on their potential to induce these enzymes (*11,12*). Using such toxicity estimates we calculated an 85% reduction in toxicity in 16 weeks as a result of the dechlorination of Aroclor 1242 by Hudson River microorganisms.

EROD induction assays were performed on the PCB extracts from live and autoclaved treatments to directly determine the toxicity reduction affected by 16 weeks of dechlorination of Aroclor 1242. A 75% reduction was observed, in good agreement with our calculations.

Extending the Range of Biodegradable Congeners

The dechlorination process also extends the range of PCB congeners that can be biologically transformed. The dechlorination of Aroclor 1254 and 1260 is particularly notable. The aerobic transformation of congeners in 1254 is limited and there is no convincing evidence for the aerobic transformation of Aroclor 1260, yet both of these Aroclors can be dechlorinated by suitable anaerobic microorganisms.

A sequential anaerobic-aerobic treatment system should also lead to greater mineralization of a PCB mixture than would aerobic treatment alone. Many of the more chlorinated biphenyls that are reportedly aerobically degraded are in fact only transformed, often merely to hydroxylated chlorobiphenyls. However, an anaerobic pretreatment would remove the chlorines that limit aerobic mineralization.

Biotreatment Scenarios

Several biotreatment systems employing a sequential anaerobic/aerobic sequence may be envisioned. Sediments might be treated *in situ*, although some form of containment will likely be required. Alternatively, sediments could be removed to a containment facility where greater control over conditions is possible. Sediments may or may not have to be inoculated with dechlorinating organisms. Soils would likely have to be slurried in a containment facility and inoculated with appropriate microorganisms to effect dechlorination. The aerobic treatment step would require some form of mixing or aeration.

Site Assessment

While the details of a sequential anaerobic/aerobic biotreatment system for the destruction of PCBs have not yet been worked out, it is possible to appraise the likelihood that a particular site can be treated in this manner. A particular site would have to be evaluated for the following:

1) The presence of dechlorinating microorganisms.
2) *In situ* dechlorination and dechlorination patterns.
3) Sediment type.
4) Nutrients / organic carbon.
5) Presence of inhibitors.
6) Bioavailability of the PCBs.

The presence of dechlorinating microorganisms.
If dechlorinators are absent the sediment or soil will have to be inoculated. This might be accomplished by adding laboratory grown microorganisms or, if in a containment facility, by simply mixing with a second sediment that does contain dechlorinators.

In situ dechlorination and dechlorination pattern.
In some cases dechlorination may have already proceeded to the point where only aerobic treatment is needed. In some cases the particular dechlorinators present may have limited dechlorination capabilities. For example, they may remove only *meta* chlorines or have limited activity on some tri- and tetrachlorobiphenyls. Then it may still be desirable to add a different dechlorinating microorganism. If no dechlorination has occurred, it may be because dechlorinators are absent or because inhibitors are present. Appropriate bioassays can distinguish between these possibilities.

Sediment type.
Oxygen diffuses much more slowly through fine grained sediments so that anaerobic conditions are more likely to develop. Anaerobic conditions are of course necessary for dechlorination to occur.

Nutrients / organic carbon.

There must be enough substrate initially present in the sediments to deplete oxygen in the sediments and consume other electron acceptors such as sulfate which inhibit dechlorination. It is still unclear what substrates are required to support the dechlorinators. Different dechlorinators may have different requirements.

Presence of inhibitors.
Bioassays may be conducted to determine if dechlorination of PCBs can occur in a given soil or sediment. We have encountered some sediments which do not support dechlorination, probably because of high levels of heavy metals.

Bioavailability of the PCBs.
The PCBs can only be dechlorinated and degraded if they are available to the microorganisms. Bioassays may also be conducted to determine bioavailability.

Research Needs

Areas requiring further research are :

1) Environmental rates / time course
2) Concentration effects
3) Enhancement of activity
4) Propagation / Identification of dechlorinators
5) Factors affecting bioavailability
6) Suitable aerobic microorganisms for an anaerobic / aerobic treatment sequence

Environmental rates / time course.
When the evidence for environmental dechlorination of PCBs was first presented, it was assumed to have been a slow continuous process taking decades to reach the extent of dechlorination observed. In laboratory experiments we have achieved the same extent of dechlorination within a few weeks. It is therefore important to determine whether PCB dechlorination *in situ* was a relatively rapid event of short duration (and has since stopped), or is in fact a continuous process. The answer to this question has important implications for both predicting the environmental fate of PCBs in anaerobic environments and in its implications for *in situ* bioremediation.

Concentration effects.
A pronounced concentration effect was observed in our laboratory experiments; no detectable dechlorination occurred within 16 weeks at a concentration of only 14 ppm of Aroclor 1242. Some environmental samples at comparable concentrations, however, do show evidence of dechlorination. It is important to determine if *in situ* dechlorination of low concentrations does occur and at what rate.

Enhancement of activity.
Ways to increase dechlorination rates will be important to developing a treatment system. Areas include determining best supporting substrates, increasing bioavailability, and counteracting inhibitory substances. It will be necessary to enhance aerobic activity by providing oxygen or aerating in some way.

Propagation / identification of dechlorinators.
Before dechlorinating microorganisms can be grown in adequate numbers to use in inoculating a sediment or soil to be treated, it will be necessary to determine how to propagate them in the absence of PCBs. Identification and isolation of the dechlorinators will aide this effort, and also allow whole new sets of experiments aimed at better understanding the dechlorination process itself.

Factors affecting bioavailability.
It is a common concern in developing biological treatment systems for poorly water soluble

compounds that rates may be inadequate and target levels not be achieved because of limited bioavailability of the compounds to the microorganisms. Even among the relatively few sediments and industrial sludges we have examined so far, the bioavailability of PCBs apparently varies widely. A better understanding of the reasons for these differences between sediments and sludges may lead to ways to increase bioavailability. It should be noted also that the generally longer time scales typical for anaerobic dechlorination may be an advantage when desorption from sediments is slow.

Suitable aerobic microorganisms for an anaerobic / aerobic treatment sequence. In the most complete dechlorination we have observed, PCBs substituted at only the *ortho* positions accumulate. It is therefore important to obtain strains capable of degrading these compounds. A serious problem with the aerobic degradation of PCB mixtures is that the PCBs are actually cometabolized and the pathway must first be induced by the addition of biphenyl. However, some strains capable of growth on monochlorobiphenyls are known. The high levels of 2-CB that are produced by the dechlorination process may induce such strains to cometabolize the remaining congeners.

References

1. Brown, J.F., D.L. Bedard, M.J. Brennan, J.C. Carnahan, H. Feng, and R.E. Wagner (1987a). Polychlorinated biphenyl dechlorination in aquatic sediments. *Science*, **236:** 709-712.

2. Brown, J.F., R.E. Wagner, D.L. Bedard, M.J. Brennan, J.C. Carnahan, R.J. May, and T.J. Tofflemire (1984). PCB transformations in upper Hudson sediments. *Northeast. Environ. Sci.*, **3:** 167-179.

3. Brown, J.F., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan, and R.J. May (1987b). Environmental dechlorination of PCBs. *Environ. Toxicol. Chem.*, **6:** 579-593.

4. Chiou, C.T., L.J. Peters, and V.H. Freed (1979). A physical concept of soil-water equilibrium for non-ionic organic compounds. *Science*, **206:** 831-832.

5. Dolfing, J. (1990). Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. *Arch. Microbiol.*, **153:** 264-266.

6. Mohn, W.W. and J.M. Tiedje (1990). Strain DCB-1 conserves energy for growth from reductive dechlorination coupled to formate oxidation. *Arch. Microbiol.*, **153:** 267-271.

7. Nies, L. and T.M. Vogel (1990). Effects of organic substrate on dechlorination of Aroclor 1242 in anaerobic sediments. *Appl. Environ. Microbiol.*, **56:** 2612-2617.

8. Ogram, A.V., R.E. Jessup, L.T. Ou, and P.S.C. Rao (1985). Effects of sorption on biological degradation rates of 2,4-Dichlorophenoxyacetic acid in soils. *Appl. Environ. Microbiol.*, **49:** 582-587.

9. Quensen, J.F., III, S. A. Boyd, and J.M. Tiedje (1990). Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Appl. Environ. Microbiol.*, **56:** 2360-2369.

10. Quensen, J.F., J.M. Tiedje, and S.A. Boyd (1988). Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. *Science*, **242:** 752-754.

11. Safe, S. (1987). Determination of 2,3,7,8-TCDD toxic equivalent factors (TEFs): Support for the use of the in vitro AHH induction assay. *Chemosphere,* **16:** 791-802.

12. Sawyer, T.W. and S. Safe (1982). PCB isomers and congeners: induction of aryl hydrocarbon hydroxylase and ethoxy resorufin o-deethylase activities in rat hepatoma cells. *Toxicol. Lett.,* **13:** 87-93.

13. Suflita, J.M., A. Horowitz, D.R. Shelton, and J.M. Tiedje (1982). Dehalogenation: A novel pathway for the anaerobic biodegradation of haloaromatic compounds. *Science,* **218:** 1115-1116.

Table 4.2.1.

Maximal observed dechlorination rates (means with standard deviations) of the Aroclors tested for microorganisms collected from the two sites. Significant differences between rates (Least Significant Difference test, 0.05 confidence level) are indicated by different capital letters next to means (From Quensen et al., 1990).

Site	Aroclor	Rate (µg atoms Cl$^-$ removed/g sediment week)	Time Period (weeks)	Percent m & p Cl removed
HR	1242	0.31^{ab}(0.03)	0-8	85[c]
	1248	0.34^{a} (0.01)	0-8	75[c]
	1254	0.22^{c} (0.02)	0-8	63[d]
	1260[a]	0.00^{d} (0.03)	0-25	0[d]
	1260[b]	0.04^{e} (0.005)	16-24	15[e]
SL	1242	0.30^{b} (0.02)	0-4	46[f]
	1260	0.21^{c} (0.01)	12-16	19[f]

[a] serum bottle experiment, sediments collected 1/88
[b] serum tube experiment, sediments collected 8/88
[c] after 12 weeks
[d] after 25 weeks
[e] after 50 weeks
[f] after 16 weeks

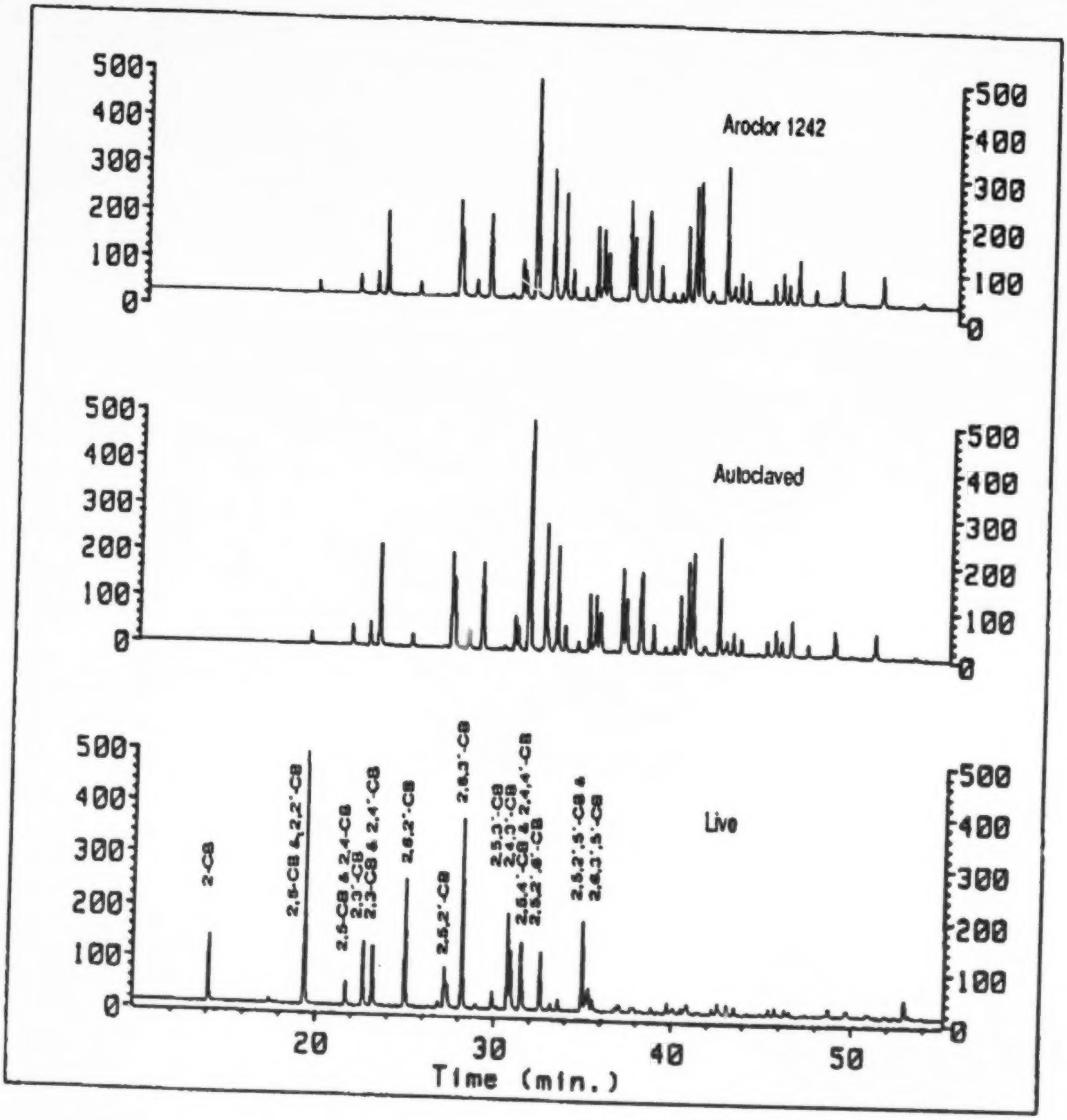


Figure 4.2.1. Capillary gas chromatograms showing the anaerobic dechlorination of 700-ppm Aroclor 1242 after 16 weeks of incubation

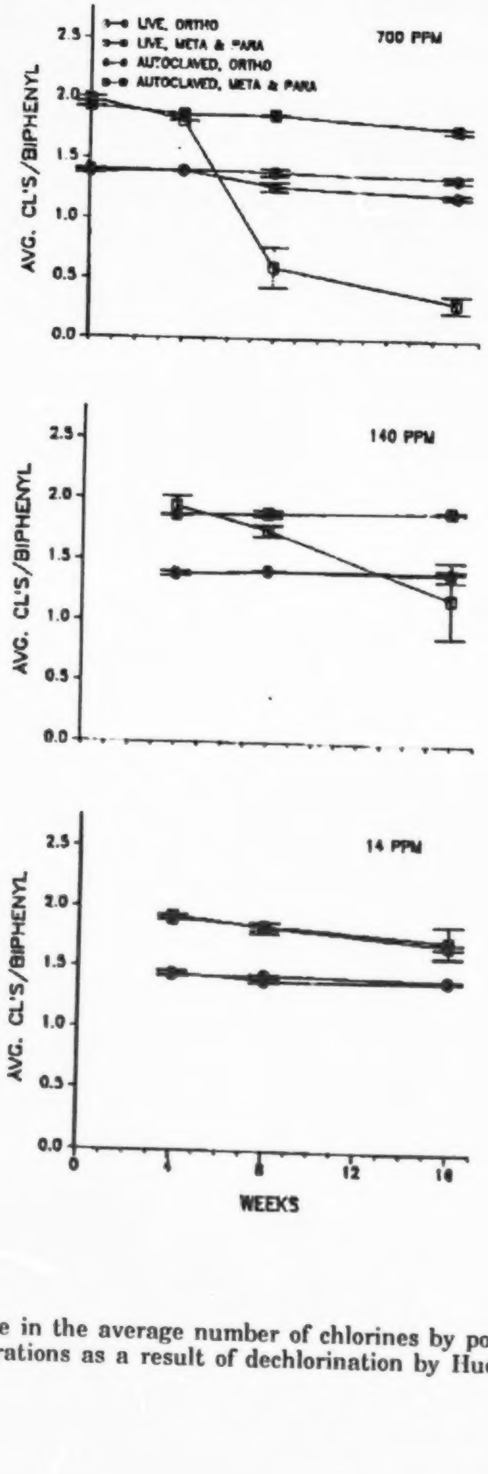


Figure 4.2.2. Decrease in the average number of chlorines by position at three Aroclor 1242 concentrations as a result of dechlorination by Hudson River microorganisms

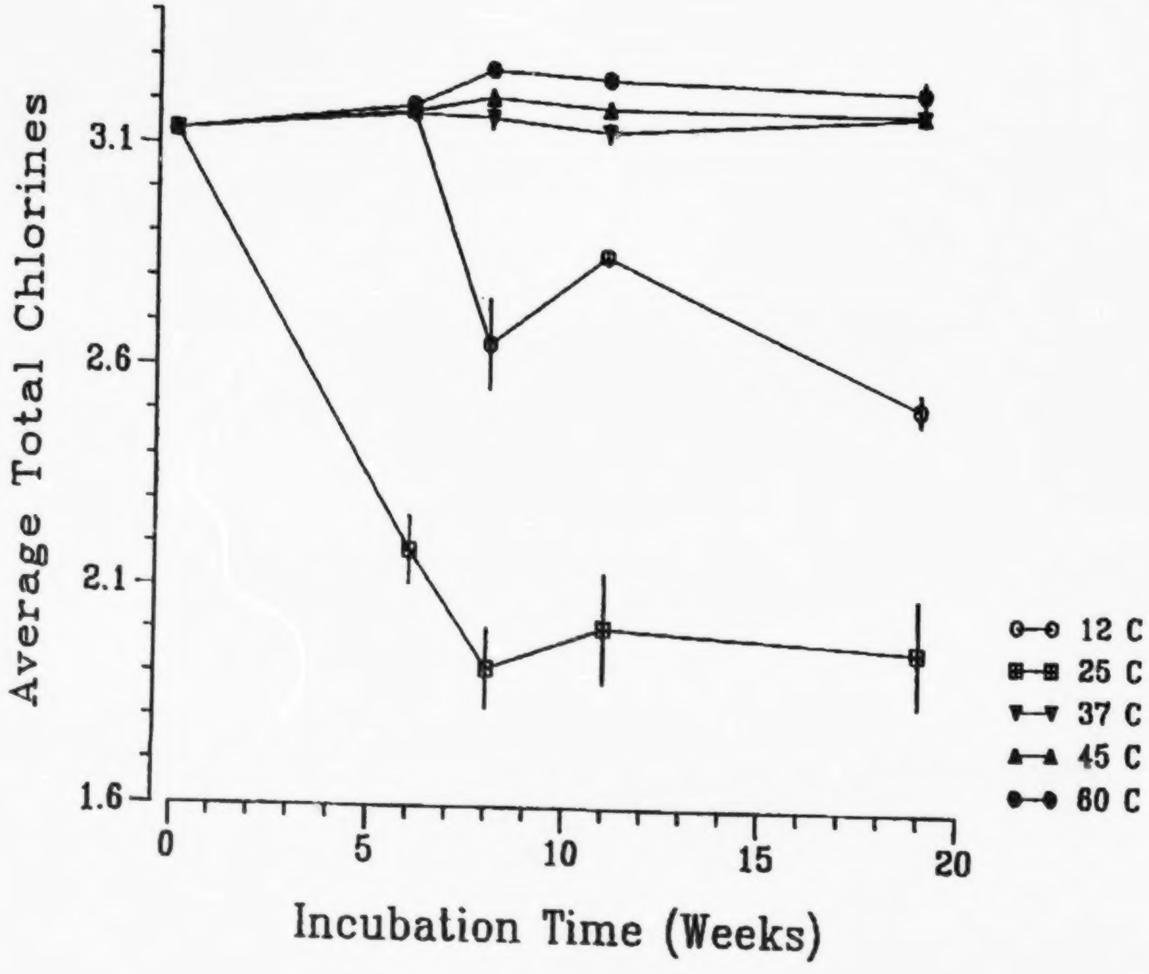
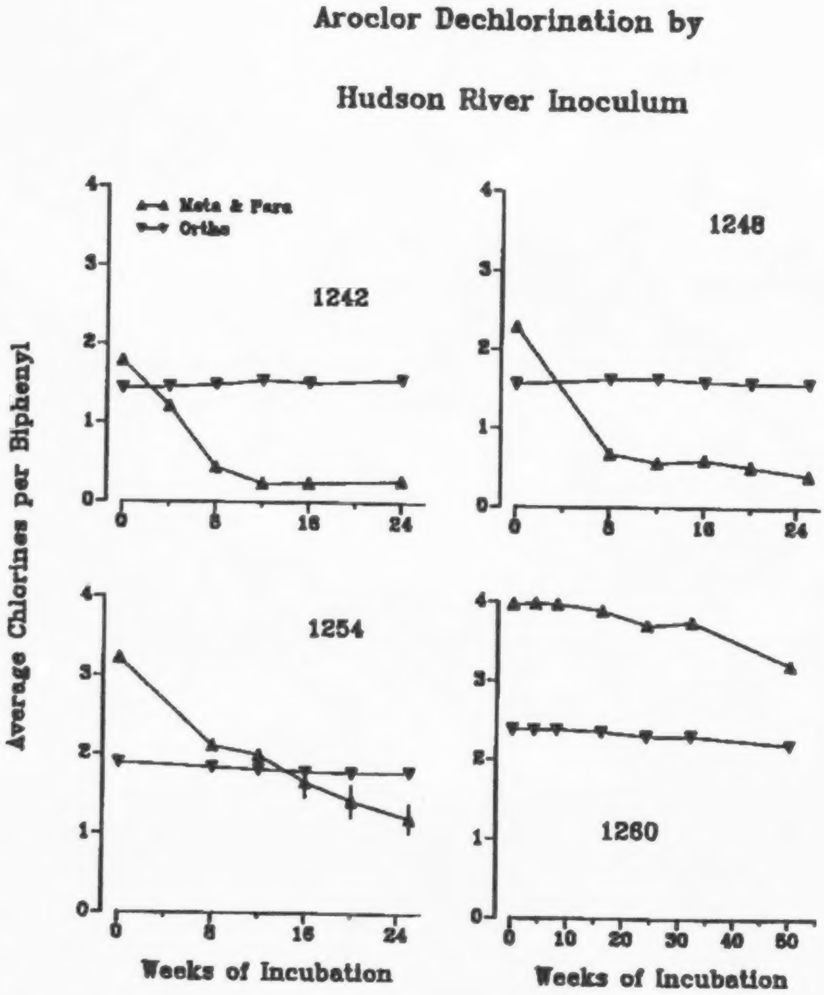


Figure 4.2.3. Effect of incubation temperature of the dechlorination of Aroclor 1242 by Hudson River microorganisms


Aroclor Dechlorination by

Hudson River Inoculum

Figure 4.2.4 Decrease in the average number of chlorines for four Aroclors as a result of dechlorination by Hudson River microorganisms

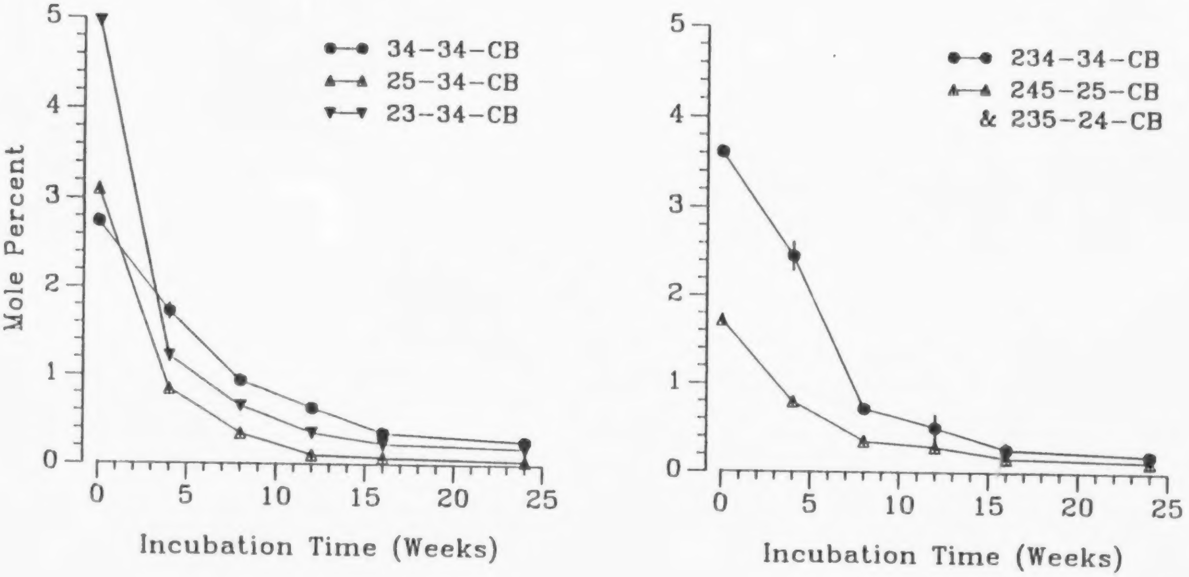


Figure 4.2.5. Comparison of the dechlorination rates of 3,3',4,4'-CB, 2,3,3',4,4'-CB, and selected tetra- and penta- CBs present in Aroclor 1242

4.3 Dechlorination and Biodegradation of Chlorinated Biphenyls in Anaerobic Sediments

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Polychlorinated biphenyls were thought to be highly resistant to biodegradation, especially in anaerobic environments due to their thermodynamic stability. The results of earlier studies reinforced this postulation. Although partial degradation of some monochlorobiphenyls was observed in some cases, breakdown by facultative anaerobes could not be excluded.

Recently, our laboratory reported anaerobic biodegradation of lightly-chlorinated PCB congeners in the laboratory by mixed cultures obtained by Aroclor 1221 enrichment of Hudson River sediments. A comparison of congener patterns in the deeper sediment layers of the Hudson River with those of the PCBs presumed to have been discharged into the river (Aroclor 1242), also led to speculation that PCBs were anaerobically dechlorinated (*1*). This hypothesis was later disputed owing to several quantitative problems (*2*). Quensen *et al.* (*3*), however, have shown unambiguous evidence for anaerobic microbial dechlorination in the laboratory with Hudson River sediments. With Aroclor 1242, they found the accumulation of lightly chlorinated, mostly *o*-substituted congeners as a result of dechlorination of *m*- and *p*-chlorines. However, they failed to find any loss of chlorobiphenyls on a molar basis.

Our investigation of anoxic Hudson River sediments showed no sign of dechlorination, especially for highly chlorinated congeners. Rather, lightly chlorinated congeners in ambient sediment PCBs exhibited significant decreases. PCBs in this study consisted mainly of lightly-chlorinated congeners. Therefore, we undertook an investigation with a mixture with greater proportions of highly chlorinated congeners and a single hexachlorobiphenyl congener to determine whether similar degradation also occurred and measure the rate. Six different concentrations ranging from 100 to 1500 ppm on a sediment dry-weight basis using Aroclor 1242 were used.

PCB-free sediments, contaminated with Aroclor 1242 at 100, 300, 500, 800, 1200, and 1500 ppm on a sediment dry weight basis and enriched with 1000 ppm biphenyl, were made into slurries by adding 20 ml of a cystine sulfide-reduced synthetic medium. They were autoclaved and then inoculated with the supernatant of Hudson River sediment slurries (0.5 ml) except for the controls for each concentrations and incubated in triplicate under N_2 atmosphere. Experiments with the single congener 2,3,4,2',4',5'-hexachlorobiphenyl was also set up in the same manner at 300 ppm. The sediment PCBs were extracted and analyzed by GC (Hewlett-Packard 5840A) with a Ni-63 electron capture detector and 50 m capillary column (DB-5).

The first analysis Aroclor 1242 after a 3-month incubation clearly demonstrated dechlorination and their dependence on the total Aroclor 1242 concentrations; the sediments with the initial concentrations of 300 and 500 ppm showed dramatic changes in congener patterns with highly significant accumulation of mono-, di- and some tri-chlorobiphenyls and concomitant decrease of most congeners with three or more chlorines. Out of 39 major peaks in the gas chromatogram comprising about 98% of the total PCB, 10 (2; 2,2'+2,6; 2,4+2,5; 2,3'; 2,4'+2,3; 2,6,2'; 4,4'+2,4,2'; 2,4,3'; 2,4,4') showed significant increases after 3 months.

The accumulation of dechlorination products relative to the control was highest in the congeners with *ortho*-substituted chlorines (2-, 2,2'-, 2,6,2-chlorobiphenyls). In absolute concentrations, 2,2'-, 2,4', and 4,4'+ 2,4,2' exhibited the highest increase.

Although the congener profile also changed in sediments with 100 and 800 ppm Aroclor after 3 months, a t-test showed no statistically discernable difference for most individual congeners. However, the difference became highly significant at 4.5 and 6 months. Dechlorination appeared to be inhibited at high concentrations, since at 1200 and 1500 ppm no change was evident even after 6 months.

Dechlorination at different substitution positions reflected the concentration dependence of overall dechlorination. *m*-Chlorine was most readily dechlorinated; an average number of this chlorine per biphenyl (0.98) decreased about 75% in 6 months in the 300 ppm sediments. *p*-Chlorine (0.88) was much slower at about 20%. However, the average number of *o*-chlorine did not appear to change.

Despite such extensive dechlorination, no significant decrease in the total molar concentration of the mixture was found by 6 months. These results indicate that no biodegradation beyond dechlorination has taken place.

The single congener 2,3,4,2',4',5'-hexachlorobiphenyl (300 ppm) was investigated using sediments reduced biologically or the same sediments further reduced with cystine sulfide. These sediments were incubated under CO_2 atmosphere with and without biphenyl enrichment. At 3 months, the sediments with cystine sulfide exhibited an extensive dechlorination, yielding daughter congeners with fewer chlorines. However, the total molar concentration of chlorobiphenyls showed no significant change. In the sediments which were not reduced chemically, the parent congener was recovered quantitatively with no dechlorination.

At 9 months, however, all treatments showed dechlorination. The first dechlorination product was 2,5,2'4'5'-pentachlorobiphenyl, which was further dechlorinated in the next step to 2,4,2',5'- or 2,4,2',4'- + 2,2'4'5'-tetrachlorobiphenyl. These products were then dechlorinated mostly to 2,2'5'-trichlorobiphenyl, with a small amount of 2,2'4'-trichlorobiphenyl also produced. They were then converted to 2,2-dichlorobiphenyl. This dechlorination pathway appeared to be the same for all incubation conditions. In all treatments, the chlorination of products decreased with time.

In summary, polychlorinated biphenyls (Aroclor 1242) were dechlorinated in anaerobic sediments by indigenous microbial populations from Hudson River sediments when incubated with biphenyl enrichment under N_2 atmosphere. *m*- and *p*-Chlorines were most readily dechlorinated, but *o*- were not. The dechlorination rate was concentration-dependent; it was fastest at a sediment PCB concentration of 300 ppm and slower at lower (100 ppm) and higher (500 and 800 ppm) concentrations. At 1200 and 1500 ppm, no sign of dechlorination was observed after 6 months. As a result of dechlorination, mono-, di- and some tri-chlorobiphenyls increased with concomitant decreases in highly chlorinated congeners. Anaerobic incubation of the single congener 2,3,4,2',4',5'-hexachlorobiphenyl produced daughter congeners with 2 - 5 chlorines with the degree of chlorination decreasing with time. The relative concentration of dechlorination products of the hexachlorobiphenyl appeared to vary with incubation conditions. Total molar concentration of the parent compound and its dechlorination products did not appear to change at 9 months.

References

1. Brown, J. F., Jr., *et al.* (1987). *Science*, **236:** 709.

2. Brown, M. P., B. Bush, G-Y. Rhee, and L. Shane (1988). *Science*, **240:** 1674.

3. Quensen III, J. F., J. M. Tiedje, and S. A. Boyd (1988). *Science*, **242:** 752.

4.4 PCB Dechlorination in the Sheboygan River, Wisconsin

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The Sheboygan River in Wisconsin flows into Lake Michigan at the city of Sheboygan, located 90 km north of Milwaukee. Due to the high concentrations of PCBs in the river sediment, the Sheboygan River and Harbor area has received national attention. The main source of contamination was from a die casting plant located in the Village of Sheboygan Falls. The contamination source area is about 22 km upstream from the mouth of the river.

Hydraulic fluids containing PCBs were used by the die casting plant from 1959 to 1971 (*11*). Apparently, a large fire occurred at the plant prior to 1959 that was caused by combustion of the hydraulic fluids then in use. Fluids containing PCBs were subsequently put in use because of their fire resistance. Based on interviews and available records, a product called Pydrol F9 was used between 1959 and 1969 and a product called Chemtrend HF30 was used between 1970 and 1971. Pydrol F9 contains Aroclor 1248, while Chemtrend HF30 contains mostly Aroclor 1254 with a small percentage of Aroclor 1248. In 1971 the use of hydraulic fluids containing PCBs ceased.

Material from the plant (oil soaked rags, hoses and other refuse) and soil from around the plant was used to construct a low dike at the edge of the Sheboygan River. The dike sloped at a 45 degree angle to the river, so erosion of the diked material into the river occurred relatively easily (*11*). Concentrations of PCBs in the soil samples were as high as 120,000 µg/g. The dye casting plant is the only known major source of PCBs to the river, therefore, the congeners deposited in the sediments were most likely the components of Aroclor 1248 and 1254.

In an article in *Science* it was reported that biological reductive dechlorination of PCBs was occurring in Hudson River sediments. There was evidence that anaerobic dechlorination was also occurring in other aquatic sediments, including Sheboygan River sediments (*4*). The Sheboygan evidence was based on observations of chromatograms obtained from the U.S. Army Corps of Engineers.

As a result of the published reports that degradation could occur and because of new analytical capabilities to do congener specific PCB analysis, research was begun to examine the distribution of PCB congeners in the Sheboygan River sediment and to determine whether anaerobic dechlorination may be occurring. The intent was to determine the congener distribution in Sheboygan River sediment and assess whether some transformations had occurred. Results of congener distributions in Sheboygan River sediment relative to distributions in Aroclors will be summarized below as well as information on the occurrence of "toxic" congeners. Finally, a summary of evidence so far to degrade PCBs in the laboratory using bacteria from Sheboygan River sediments will be made.

Total PCB concentrations ranged from 1586 µg/g found downstream from the source to 0.04 µg/g above the source (considered to represent background levels). Although there is considerable variation in the sediment PCB concentrations, in general, the values decreased with distance downstream from the source. Highest PCB concentrations were found in areas of sediment deposition in the river. In the individual cores, the top segment of core (0-15 cm) and the bottom segment of core (45-60 cm) had relatively low concentrations of PCBs. The highest concentrations were found in the 15-45 cm segments.

Sediment samples were also analyzed for PCB congeners using high resolution gas chromatography. Samples containing total PCB concentrations greater than 50 µg/g appeared to be enriched with the lower chlorinated congeners whereas those with less than 50 µg/g PCBs were not. Samples containing 50 µg/g or more PCBs had significantly higher concentrations of mono- and di- chlorinated congeners when compared to Aroclors 1248 and 1254 which were originally introduced into the river. Using a multivariate ANOVA statistical test, samples containing greater then 50 µg/g were found to be statistically different from Aroclor 1248, Aroclor 1254 and an equal parts mixture of Aroclors 1248 and 1254 (p values were < 0.05). In sediment samples containing PCB concentrations less than 50 µg/g, the homolog patterns were more similar to the patterns of the Aroclor 1248 and 1254 than the more contaminated sediments.

The most prominent congeners in the sediments with total PCB concentrations greater than 50 µg/g were (IUPAC #) 5/8, 17, 16/32, 47/48, and 28/31. Relative to the original Aroclors, particularly high concentrations of congeners 5 and 8 were seen (congeners 5 and 8 coelute). To confirm the presence of congeners 5/8, six of the samples containing high concentrations of PCBs (342.8 µg/g on average) and high concentrations of congener 5/8 (43.7 percent on average) were analyzed using an electron impact gas chromatography mass spectrometer. All samples contained high concentrations of dichlorinated congeners. In samples containing less than 50 µg/g of PCBs, the most prominent congeners were similar, but their weight percents were generally reduced.

The results from the sediment analyses indicate that the PCB congeners and their respective weight percentages in sediments with high PCB concentrations are significantly different from the Aroclors originally introduced into the river. Although physical-chemical processes such as sediment-water partitioning are important in determining the distribution of congeners in sediments, it is unlikely that it is the dominant process influencing the distribution of congeners. Sediment-water partition coefficients generally increase with molecular weight and thus an enrichment of the higher chlorinated congeners in the sediments, not the lower chlorinated congeners as observed in the sediments, would be predicted.

Diffusion of congeners out of the sediment and into the water is slow relative to sedimentation rates and is inversely related to partition coefficients (*7,8*). Therefore, a distribution enriched in the higher chlorinated PCBs would be predicted (opposite of what was observed in this study).

Another possibility to account for the change in congener patterns is abiotic chemical reactions. PCBs have been shown to undergo abiotic reductive dechlorination in the laboratory; however, the conditions in the laboratory (high temperatures, excess base, and the presence of a catalyst) are considerably different from those in the environment (*3*). In general, it is thought that there are very few abiotic pathways which completely mineralize organic contaminants (*1*).

It is possible, however, that the Aroclors undergo biological dechlorination. Recent work by Quensen *et al.*(*9*), Chen *et al.* (*6*), Rhee *et al.* (*10*), and Brown *et al.* (*4,5*) suggest that PCBs can undergo anaerobic microbial degradation. Several results in this study suggest such a process.

First, there is a shift in the congener pattern from the higher chlorinated congeners to the lower chlorinated congeners as observed by Quensen *et al.* (*9*) in a laboratory experiment and as noted by Brown *et al.* (*4*) in a field study of Hudson River sediments. This enrichment in lower chlorinated congeners cannot be accounted for by physical-chemical partitioning relationships or diffusion processes.

Second, there appears to be a structural selectivity as to which congeners are depleted in the sediment. Congeners containing chlorines in the *ortho* position are enriched, whereas congeners containing chlorines in the *meta* and *para* position are depleted. This is consistent with the results obtained by Quensen *et al.* (*9*) and Brown *et al.* (*4*) in their anaerobic microbial dechlorination work.

Third, several congeners are found in abundance that would not be expected based on physical-chemical partitioning relationships or on the original weight percentages present in the Aroclor mixtures. In sediment samples with concentrations of PCBs above 50 µg/g, congeners that were significantly enriched are 5/8, 19, 17, 24/27, 16/32, 26, and 47/48. Congeners that were significantly depleted are 18, 28/31, 52, 44, 70/76, 66/95, 56/60, 101, 77/110, 132/153/105, and 138/163. These changes are comparable to Brown *et al.*'s (*5*) findings.

Fourth, the concentration of PCBs in the sediment appears to be important. Microbial degradation is often restricted to areas of high substrate concentrations. For example, toluene, xylene, and naphthalene are metabolized by bacteria at high concentrations but not at low concentrations (*2*). Threshold concentrations exist for many contaminants and are the minimum concentration of a chemical which is needed to support growth of a microbial population (*2*). Below the threshold concentration, additional energy sources must be found to support growth of the population, since the organism is no longer able to completely mineralize the substrate. Based on the different chromatographic patterns seen for high and low concentrations of PCBs in the Sheboygan River, it may be that a threshold concentration exists for the anaerobic dechlorination of PCBs.

To confirm that microbial processes are actually responsible for degrading PCBs, laboratory experiments have been conducted similar to those reported by Quensen *et al.* (*9*) and Rhee *et al.* (*10*). Using bacteria extracted form Sheboygan sediments, degradation was attempted using growth medium and anaerobic conditions suitable for microbial dechlorination of PCBs. However, to date no dechlorination has been observed in the experiments. The reasons for the lack of dechlorination activity is not clear, but it is suspected that the conditions that favor degradation are very complicated (e.g., may involve very precise Eh conditions and may involve several different species or strains or organisms) and may be difficult to consistently reproduce in the laboratory.

Finally, Sheboygan sediments have been analyzed for the presence of non-*ortho* or coplanar PCBs. These congeners are believed to be the most toxic (at least in terms of dioxin like toxic properties), but generally coelute with other congeners using capillary column gas chromatography. A multidimensional ("heart cutting") gas chromatograph that uses two high resolution columns in series was used to separate coeluting congeners. Results to date indicate that several congeners of toxicological interest are found in sediment samples, albeit at low concentrations. Congeners 118, 105 and 77 were detected in 83 percent of the samples analyzed, at average concentrations of about 0.25, 0.06, and 0.04 µg/g, respectively. The average composition of these congeners was 0.13, 0.03 and 0.02 percent, respectively. Congeners 81, 114, 167, 126 and 169 were also detected in some of the samples, all at concentrations less than 0.03 µg/g. While the concentrations of these congeners are low relative to total concentrations of PCBs or to the congener they coelute with, the fact that they are present may be important toxicologically. Research is ongoing in this area.

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References

1. Alexander, M (1981). Biodegradation of chemicals of environmental concern. *Science*, **211:** 132-138.

2. Alexander, M (1985). Biodegradation of organic chemicals. *Environ. Sci. Technol.*, **18:** 106-111.

3. Boyer, S.K., J. McKenna, J. Karliner, and M. Nirsberger (1985). A mild and efficient process for detoxifying polychlorinated biphenyls. *Tetrahedron Letters*, **26:** 3677-3680.

4. Brown, J.F., D.L Bedard, M.J. Brennan, J.C. Carnahan, H. Feng, and R.E. Wagner (1987a). Polychlorinated biphenyl dechlorination in aquatic sediments. *Science*, **236:** 709-712.

5. Brown, J.F., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan, and R.J. May (1987b). Environmental dechlorination of polychlorinated biphenyls. *Environ. Toxicology and Chemistry*, **6:** 579-593.

6. Chen, M., C.S. Hong, B. Bush, and G.Y. Rhee (1988). Anaerobic biodegradation of PCBs by bacteria from Hudson River sediments. *Ecotoxicology and Environ. Safety*, **16:** 95-105.

7. DiToro, D.M., J.M. Jeris, and D. Clarcia (1985). Diffusion and partitioning of hexachlorobiphenyl in sediments. *Environ. Sci. Technol.*, **19:** 1169-1172.

8. Fisher, J.B., R.L Petty, and W. Lick (1983). Release of polychlorinated biphenyls from contaminated lake sediments: flux and apparent diffusivities of four individual PCBs. *Environ. Pollut. Series B.*, **5:** 121-132.

9. Quensen, J.F., J.M. Tiedje, and S.A. Boyd (1988). Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. *Science*, **242:** 752-754.

10. Rhee, G.Y., B. Bush, M.P. Brown, M. Kane, and L. Shane (1989). Anaerobic biodegradation of polychlorinated biphenyls in Hudson River sediments and dredged sediments in encapsulation. *Water Research*, **23:** 957-964.

11. Wisconsin Department of Natural Resources (1989). *Sheboygan River remedial action plan*. Environmental Quality Division, Madison, Wisconsin.

4.5 Anaerobic and Aerobic Biodegradation of Endogenous PCBs

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INTRODUCTION

Environmental reductive dechlorination of PCBs has been widely observed in contaminated sediments, and has recently been reviewed (*1,3*). In addition, microbial anaerobic dechlorination of PCBs in aquatic environments has been confirmed in the laboratory (*2,5*). This report will focus on recent findings involving the acceleration of dechlorination in Hudson River sediments, the dechlorination of endogenous PCB contamination, as well as the sequential anaerobic/aerobic treatment of contaminated sediments.

The acceleration of anaerobic dechlorination in Hudson River sediments was observed upon the addition of a complex nutrient mixture, surfactants, or a simple trace metal mixture. The latter result may indicate that low levels of a trace metal in the sediment may limit the rate of PCB dechlorination occurring in the environment today. Dechlorination of endogenous PCB-contamination has been observed in three different soils and sediments. This result indicates that anaerobic microorganisms have access to PCBs in even "aged" soil environments. In addition, sequential microbial treatment via anaerobic dechlorination and aerobic biodegradation has been demonstrated on such endogenous PCB contamination.

RESULTS AND DISCUSSION

Rate Enhancement

The addition of a minimal medium to the sediment slurry resulted in a dramatic increase in the observed rate of anaerobic dechlorination after 8 weeks (see Figure 4.5.1). The RAMM minimal medium contained nutrients, trace minerals, and bicarbonate (*6*). The control was autoclaved and incubated along with the samples; no change was observed in any of the heat treated controls during the experiments. The control (Figure 4.5.1A), therefore, represents the PCB distribution in the original mixture added to the sediment (70% Aroclor 1242, 20% Aroclor 1254, 10% Aroclor 1260). The sample mixed with distilled water in place of the minimal medium is shown in Figure 4.5.1B. Only slight dechlorination was observed in this experimental sample after an 8-week incubation. This is contrasted by the significant change observed in the sample to which RAMM minimal medium has been added (Figure 4.5.1C). The selective *meta*-and *para-* dechlorination observed in this sample is consistent with the environmental changes observed in the Hudson River (*4*). This result suggests that a limiting nutrient present in the RAMM medium may be restricting the rate of dechlorination in Hudson River sediments. It should be noted that at later timepoints significant dechlorination was also observed in the sediment to which no nutrients were added. These changes were similar although less extensive than the sample to which nutrients were added. Therefore, nutrient addition can decrease the lag time before activity is initiated, as well as increase the extent of dechlorination observed.

The RAMM medium was subdivided into four different components to further investigate nutrient stimulation of this PCB dechlorination activity. Individual components were added in various combinations and concentrations; results are shown in Table 4.5.1. Note that the addition of the trace metals (Zn^{+2}, Cu^{+2}, Ni^{+2}, SeO_3^{-2}, BO_3^{-3}) correlates with nearly a two-fold increase in the rate of dechlorination of 234-34-chlorobiphenyl (CB). This effect suggests that one of these trace metals, added at less than 0.02 ppm level, may represent the component that limits the PCB dechlorination in Hudson River sediments. Other agents that have demonstrated to increase the rate and/or extent of PCB dechlorination in Hudson River sediments include non-ionic high molecular weight surfactants (e.g. Triton X-705) and the addition of a complex carbon source (e.g. yeast extract or fluid thioglycollate medium with beef extract). In addition, PCB dechlorination has been observed over a broad range of temperatures (5-30°C) and PCB concentrations (20-1500 ppm).

Patterns

It has been observed that minor modifications to the RAMM medium dramatically affect the observed dechlorination pattern for Hudson River sediments. This effect is demonstrated by preferential dechlorination for 24- or 25- chlorophenyl PCB congeners in Figure 4.5.2. In Figure 4.5.2B the addition of the minimal medium (RAMM) to the sediment results in extensive dechlorination of the mixture, with corresponding large increases in the resultant 2-; 2-2-; 2-3-; 2-4-; and 26-2 chlorobiphenyl peaks. The shaded peak, 2356-245-heptachlorobiphenyl, is virtually untouched in these systems and serves as an internal reference for comparisons. In Figure 4.5.2C, the effect of the minimal medium and the reductant cysteine hydrochloride is shown.

The addition of minimal salts (Figure 4.5.2B) supports the growth of a microbial population which more readily attacks PCB congeners containing a 25-dichlorophenyl ring than a 24-dichlorophenyl ring (pattern M). Note that the congeners 25-25-; 25-4; and 25-2-chlorobiphenyl have all decreased in area while the corresponding 24-24-; 24-4-; and 24-2- chlorobiphenyls have not decreased. But the addition of reductant (Figure 4.5.2C) now supports a microbial population which prefers the 24- over the 25-dichlorophenyl groups (pattern Q). It is also possible to determine conditions which support the growth of both of these microbial populations (data not shown).

Endogenous PCBs

It is possible that biodegradation studies on soils spiked with PCBs may not provide accurate kinetic data for similar experiments on endogenous, aged PCB contamination. It has been observed with South Glens Falls dragstrip soil that aerobic biodegradation rates can be limited due to bioavailability issues (data not shown). Therefore several different contaminated soils and sediments were investigated to directly monitor the PCB dechlorination rate of the endogenous contamination.

Hudson River sediments contaminated >15 years ago have already been extensively dechlorinated in the environment (*4*). Such sediments can be even further dechlorinated by the addition of RAMM nutrients (see Figure 4.5.3B). The dechlorination rate observed is comparable to that found in spiked samples. Endogenous PCB contamination can also be dechlorinated in Woods Pond sediments (Aroclor 1260, data not shown).

The endogenous PCBs bound to dragstrip soil were also available for dechlorination via anaerobic microorganisms (see Figure 4.5.4). In this experiment, 25% by weight Hudson River sediments were added to the dragstrip soil containing RAMM medium. Again, this microbial process can successfully attack the endogenous PCB contamination. This result is particularly encouraging since this same soil demonstrated bioavailability limitations upon aerobic treatment.

Sequential Anaerobic/Aerobic Treatment

Hudson River sediments that had previously undergone environmental dechlorination were then treated by aerobic PCB-degrading organisms to demonstrate the effect of this combined process (see Figure 4.5.5). Figure 4.5.5A represents the original contamination (Aroclor 1242); Figure 4.5.5B displays a recently obtained sediment sample from the Hudson River that has been environmentally dechlorinated (>85% mono- and di-CB); Figure 4.5.5C displays the resulting chromatogram after aerobic treatment. This initial trial demonstrated >70% reduction in the PCB concentration after one day of aerobic treatment.

CONCLUSIONS

Hudson River Sediments contain anaerobic microorganisms capable of extensively dechlorinating PCB mixtures. The observed rate of laboratory dechlorination can be stimulated by the addition of trace metal mixture or detergents at low concentrations. Different dechlorination patterns observed under different experimental conditions indicate that these sediments contain a complex microbial population of different dechlorinating organisms.

Experiments on a variety of PCB contaminated soils have demonstrated that this anaerobic process will effectively attack endogenous PCB contamination. No significant rate difference was observable for endogenous or spiked PCB samples. In addition, sequential anaerobic/aerobic treatment of the PCB contamination present in Hudson River sediments have resulted in a >70% reduction in total PCB concentrations and a dramatic shift in PCB distribution to lightly chlorinated material.

REFERENCES

1. Abramowicz, D.A. (1990). **In:** *CRC Critical Reviews in Biotechnology* (eds.), G.G. Steward and I. Russell, CRC Press, Inc., in press.

2. Abramowicz, D.A., M.J. Brennan, H.M. Van Dort and E.L. Gallagher (1990). **In:** *Chemical and Biochemical Detoxification of Hazardous Waste II* (ed.), J. Glaser, Lewis Publishers, in press.

3. Bedard, D.L. (1990). **In:** *Biotechnology and Biodegradation,* (eds.), D. Kaemly, A. Chakrabarty, G.S. Omenn, Adv. Appl. Biotechnol. Series, Vol. 4, Portfolio Pub. Co., The Woodlands, TX., pp. 369-388.

4. Brown, J.F., Jr., D.L. Bedard, M.J. Brennan, J.C. Carnahan, H. Feng and R.E. Wagner (1987). *Science*, **236:** 709-712.

5. Quensen, J.F., Jr., J.M. Tiedje, and S.A. Boyd (1988). *Science*, **242:** 752-754.

6. Shelton, D.R. and J.M. Tiedje (1984). *Appl. Environ. Microbiol.*, **47:** 850-857.

TABLE 1: Effect of RAMM Components on Dechlorination Rate

Relative Dechlorination Rate	A	B	C	D
105%	+			
90	+	+		
105	+	+	+	
171	+	+	+	+
210	+	++	+	+
171	++	++	+	+
202	+	+	++	+
210	+	+	++	+
191	+	+		++

A = Phosphate Salts, Cystein, HCO_3^{-}
B = Nitrogen + Minerals ($CaCl_2$, $MgCl_2$, $FeCl_2$)
C = $MnCl_2$, MoO_4^{-2}, $CoCl_2$
D = Trace Metals (BO_3^{-3}, Zn^{+2}, Cu^{+2}, Ni^{+2}, SeO_3^{-2})

Table 4.5.1. Effect of RAMM components on dechlorination rate

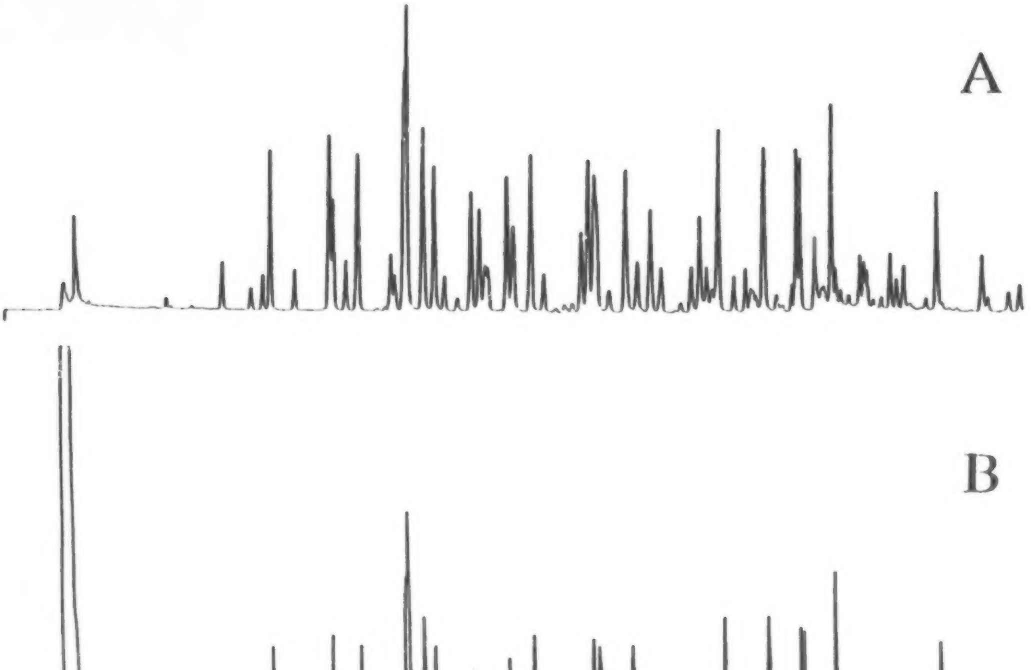

Figure 4.5.1. Acceleration of the reductive dechlorination of PCBs upon addition of nutrients (8 week timepoint). A) autoclaved control; B) includes distilled water; C) includes RAMM minimal medium. All samples contain 500 ppm PCB (70% Aroclor 1242, 20% Aroclor 1254, 10% Aroclor 1260) inoculated with sediments from the Hudson River.

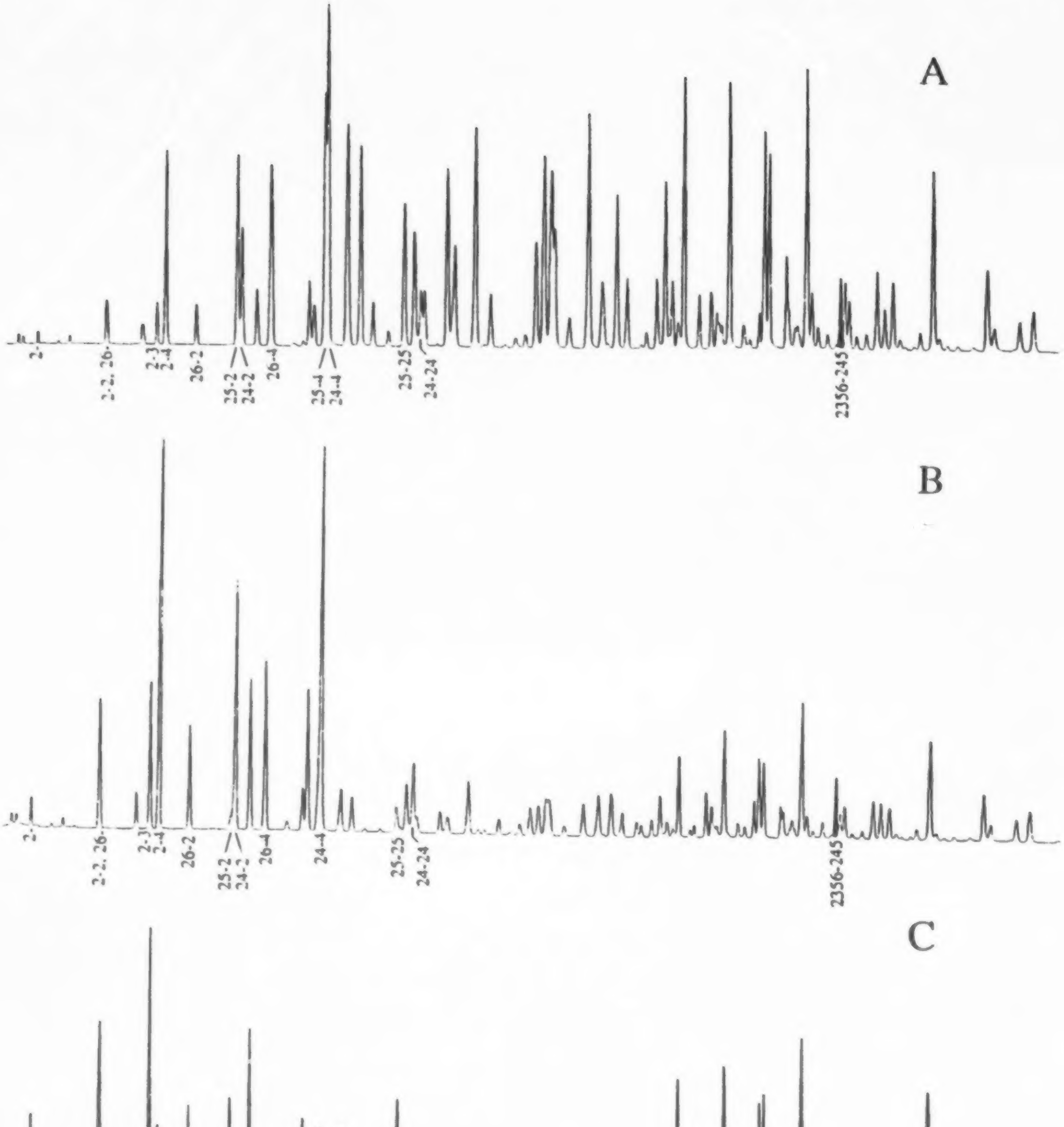

Figure 4.5.2. Dechlorination patterns observed under different conditions (18 week timepoint). A) autoclaved control; B) includes RAMM (pattern M); C) includes RAMM + cysteine hydrochloride at 1 gm/L (pattern Q)

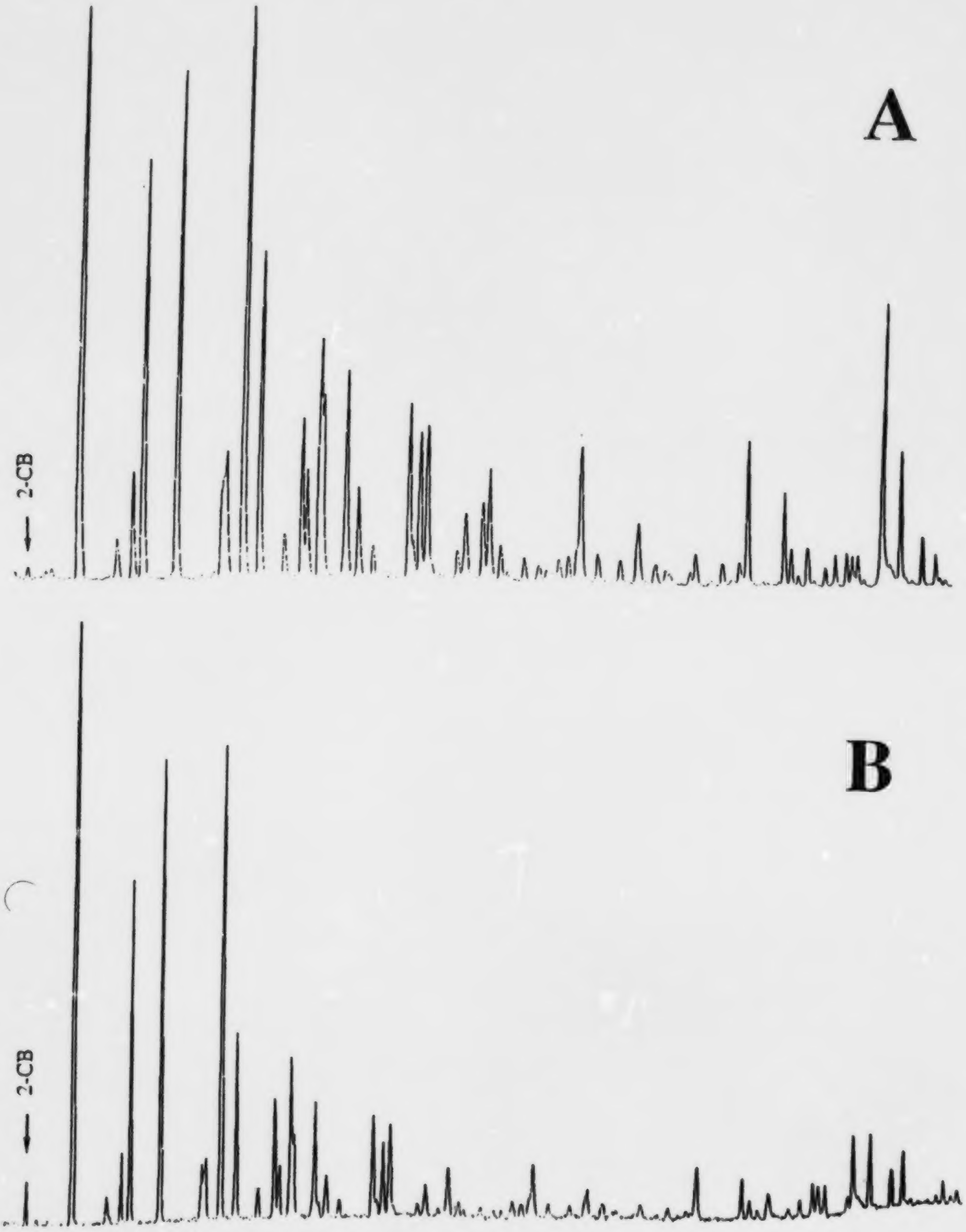

Figure 4.5.3. Dechlorination of endogenous PCB contamination in Hudson River sediments with RAMM (18 week timepoint). A) autoclaved control; B) experimental.

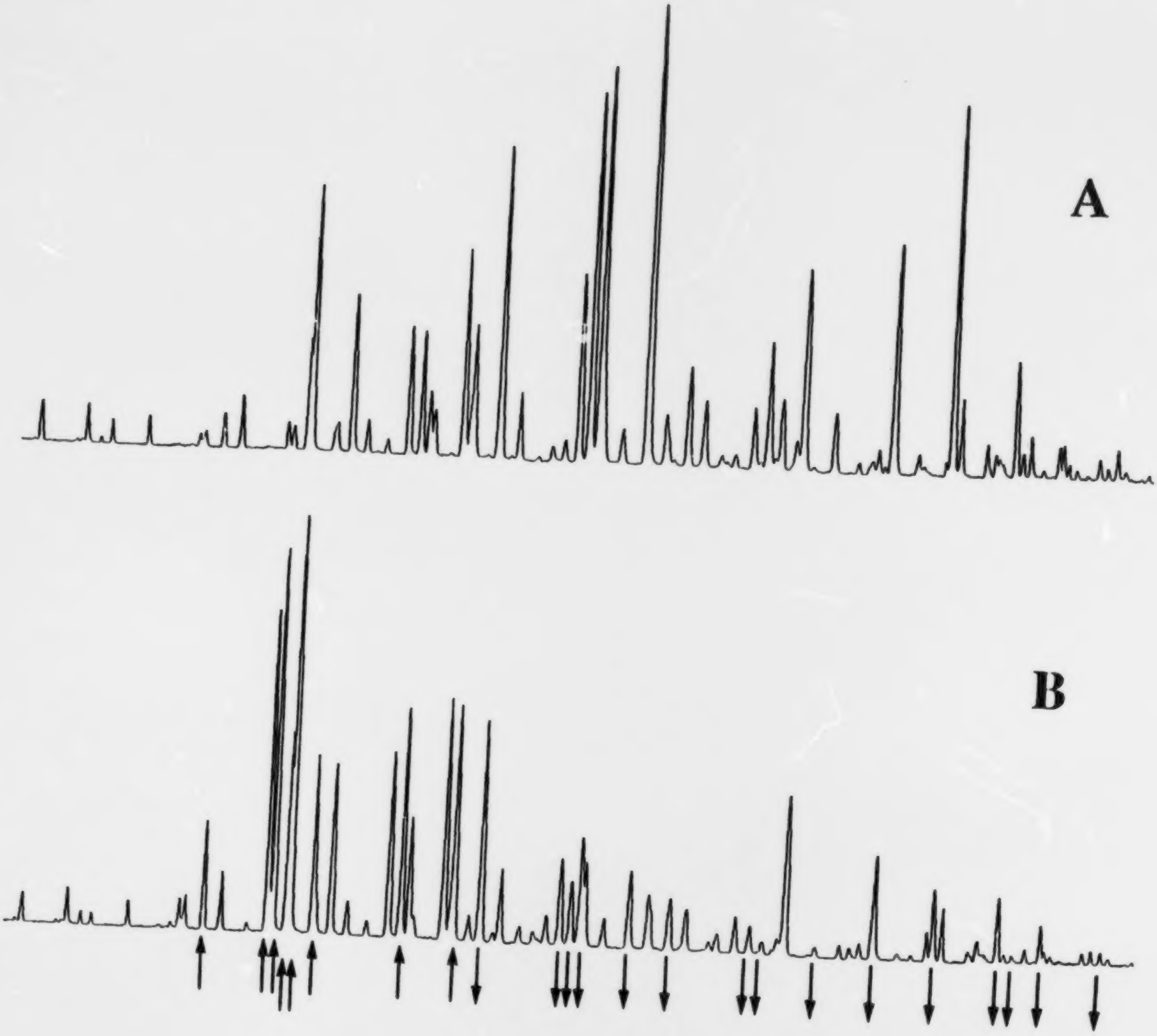

Figure 4.5.4. Dechlorination of endogenous PCB contamination in South Glens Falls soil with 25% Hudson River sediment (23 week timepoint). A) autoclaved control; B) experimental.

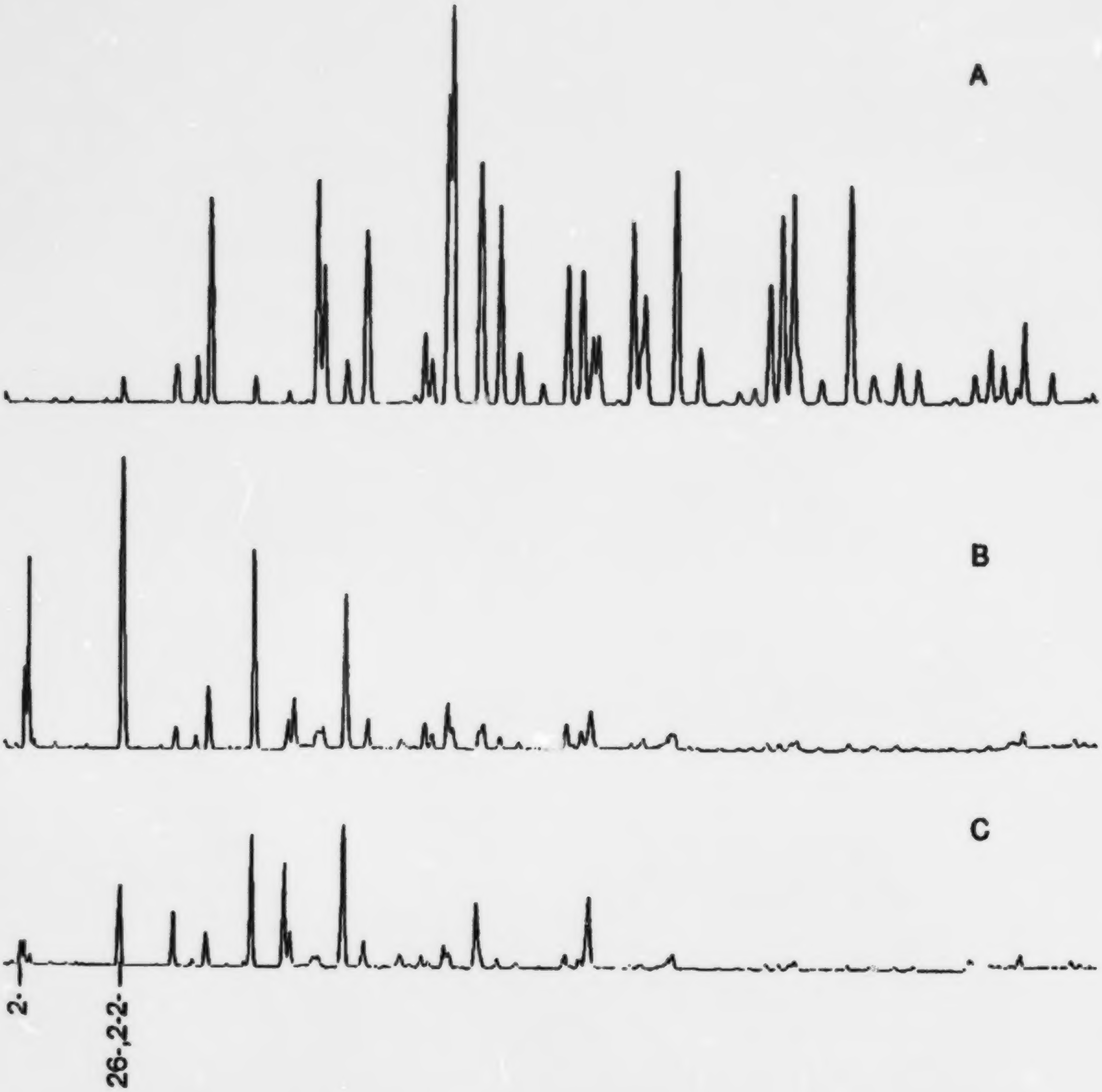

Figure 4.5.5. Sequential Anaerobic/Aerobic treatment of endogenous PCB contamination in Hudson River sediments. A) Aroclor 1242; B) environmentally dechlorinated Aroclor 1242; C) B+ aerobic treatment (1 OD cells; 1 day timepoint).

4.6 Remediation Pilot Study in the Sheboygan River, Wisconsin, USA

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The Sheboygan River and Harbor site, located approximately 55 miles north of Milwaukee, Wisconsin, was placed on the National Priorities List (NPL) in May 1986. The site includes approximately 14 miles of river and a 100-acre harbor. The river, which flows easterly toward Lake Michigan, drains 432 square miles of central Wisconsin countryside. The contaminants of concern include PCBs and various metals.

The Sheboygan River and Harbor Remedial Investigation/Feasibility Study (RI/FS) Program began in 1986. Blasland & Bouck Engineers, P.C., (Blasland & Bouck) on behalf of Tecumseh Products Company (one of three identified potentially responsible parties), developed the work plan and appropriate project plans for the investigation work efforts. Remedial Investigation field efforts, conducted in a phased approach, began in 1987. The first phase involved obtaining a number of "key" samples from the river and harbor which were subsequently analyzed for the hazardous substance list (HSL). Based on results from this first round, the contaminants of concern were confirmed to include PCBs and eight metals. During the course of the remedial investigations, approximately 250 sediment cores, three rounds of water column samples and 20 floodplain soil samples were analyzed for these contaminants.

After a preliminary screening of potentially applicable technologies for remediation of the site (if deemed necessary), it became apparent that additional information would be necessary to perform a meaningful comparative analysis of remaining technologies. This was especially true for those technologies considered to be both promising and innovative.

The results of the preliminary screening, coupled with the EPA's request to remove three sediment areas with elevated concentrations, prompted a proposal by Tecumseh to conduct an Alternative Specific Remedial Investigation (ASRI) to provide the information necessary to conduct a comprehensive feasibility study. The proposed ASRI activities fall into two distinct categories. The first consists of a pilot study to investigate the feasibility of enhancing natural biodegradation of PCBs. The process of biodegradation is believed by experts to be already occurring in the river. The second category includes various bench-scale studies of other potentially applicable technologies and additional investigative efforts to further supplement the remedial investigations. More specifically, the primary objectives of the ASRI work efforts, including the pilot study activities, are as follows:

A. Study the potential for enhancing natural biodegradation
B. Evaluate mechanical dredging in the Sheboygan River
C. Evaluate the effectiveness of in situ capping or "armoring"
D. Monitor the impact of activities on the water column
E. Conduct bench-scale studies of promising and innovative remedial technologies for site sediments

Each of these objectives is further defined in the text which follows.

Blasland & Bouck designed a pilot scale confined treatment facility (CTF) to study the effectiveness of using enhanced biodegradation for treatment of contaminated sediments removed from the river. In addition, it was determined that enhancement of biodegradation should be investigated with sediments remaining in the river. This was to be accomplished by capping or "armoring" the sediments, and then monitoring to see if the conditions for natural biodegradation could be improved.

The pilot scale CTF can accommodate approximately 2500 cubic yards (cy) of sediments removed from the river, and is constructed of structural steel sheet piling. The 14,000 square-foot structure is divided into four separate cells, two study cells and two control cells. Each cell is lined with two high density polyethlyene liners with a leak detection system in between.

Each cell has an independent discharge which exits the cell by flowing through a permeable treatment wall (PTW). This special design feature provided to study alternative means for treatment of water discharges, consists of various configurations of sand and "organic" material. Water from the four cells will be allowed to flow from top to bottom through the PTWs for cells 1 and 2, and horizontally through an unlined sheet piling wall for cells 3 and 4. The discharge from each PTW will be comparatively evaluated for treatment effectiveness. The intent of the alternative water treatment study is to identify an effective effluent treatment material for possible scale up to a full scale CTF, should this be deemed necessary. In addition to the special CTF design features mentioned above, an amendment distribution system is provided in the bottom of each cell to facilitate introduction of materials for the enhancement of biological activity.

Bench-scale biodegradation studies are currently underway at the University of Michigan, under the direction of Dr. Timothy Vogel. Dr. Vogel's efforts include work with both anaerobes and aerobes from the Sheboygan River and other PCB-contaminated sites. Research efforts are ongoing and will continue into the fall. The results from this work (and that of other researchers) should provide the information necessary to enhance the process that nature has already begun.

Sediment removal activities were designed to minimize exposure of the sediments to the air, in order to preserve the conditions necessary for the indigenous bacteria. As such, the sediments are mechanically removed utilizing a sealed clamshell. The sediments are then placed into a sealed 7-cubic yard capacity transport box. Within the river, each sediment area to be removed is surrounded with a double silt curtain system to prevent the downstream movement of materials suspended in the water column during the removal process. The silt curtain system consists of an outer geomembrane curtain and an inner geotextile curtain. Each curtain is weighted with flexible chain or cable to conform to the configuration of the river bottom.

Sediments are removed in two "passes". The first pass removes the majority of the sediment deposit, usually to the hard underlying clay. The area within the curtains is then allowed to "rest" (remain quiescent) for a minimum of 12 hours prior to conducting a second pass. The intent of the second pass is to enable removal of the settled fines to the extent possible, and provide for a "buffer" of underlying material to be removed. After all sediment is thought to have been removed from each area, the river bed is probed, and post removal sediment and water column samples are obtained for analysis.

Armoring confines the sediments in place by covering the deposits with successive layers of materials to minimize resuspension. The same silt curtain system is employed during the armoring activities. Armoring of the sediments is accomplished as follows:

A. Geotextile material is placed on the sediment deposit, extending beyond the sediment limits by five feet;
B. A 6-inch layer of run of bank material is placed;
C. Another layer of geotextile is placed;
D. Rock-filled wire cages, called gabions, are placed along the periphery of the sediment area to anchor the geotextile layers; and
E. A layer of stone is placed for ballast.

To accommodate the monitoring of biological activity under the armoring materials, a sampling port is provided in a number of the armored sediment areas. This sampling port will allow for retrieval of sediment samples every six months from underneath the armoring material. These samples will be subjected to congener specific analyses to assess PCB biodegradation.

As with any pilot study activity, monitoring of the effects before, during and after the activity is important; this pilot study is no different in this respect. River monitoring activities include daily monitoring of the water column, both upstream and downstream of the actual work area, whenever work is being conducted. Samples are obtained and total suspended solids (TSS) and turbidity determined. In addition to the daily monitoring, weekly water column samples are obtained and analyzed for PCBs (total and filtered).

Biological monitoring (both in situ caged fish studies and analysis of resident and migratory species) is also being employed. The in situ fish monitoring consists of caged fish studies (42-day exposure). Pre-construction monitoring was conducted in September 1989 (before any river activities were initiated) and removal/armoring monitoring took place in December 1989. The final set of in situ fish studies will be conducted well after all river activities are complete. This post construction study will be conducted during a similar time period as the pre-construction study to minimize the effects of water temperature.

Extensive monitoring of resident and migratory species of the Sheboygan River is already ongoing to develop an adequate data base with which to compare future fish results after the pilot study and final remedy are completed (should the latter be necessary). The fish selected for monitoring include:

A. Chinook salmon
B. Steelhead trout
C. Small-mouth bass
D. Sucker species (preferably young-of-the-year)

Collection of these fish occurs throughout the year, as appropriate.

Bench-scale study efforts include gathering additional information on the physical characteristics of the sediment and applicability of various technologies for remediation. Further physical characterization includes obtaining supplemental information for the sediments such as in situ density, particle size, affinity of PCBs for different sized particles, and Atterberg limits. Other sediment characteristics related to handling prior to treatment or for design purposes require further definition. These include settleability, dewatering ability, consolidation, and leachability.

Preliminary technology assessment and determination of applicability to the Sheboygan River and Harbor sediments will be conducted on a number of technologies. These include biodegradation (previously mentioned), a number of extraction methods, stabilization/fixation, and in situ armoring. Many of these studies are already ongoing or are to be conducted in the near future.

Sediment removal/armoring activities are anticipated to be complete by the end of 1990. As previously mentioned, it is hoped that biodegradation studies within the CTF will be initiated by late fall. The other bench-scale treatability studies and the sediment characterization work will be presented in a final ASRI report to the reviewing agencies. It is anticipated that this ASRI report will be available by the end of 1991. The final Feasibility Report for the site will be developed thereafter.

5 POLYCYCLIC AROMATIC HYDROCARBONS

5.1 The Use of a *Mycobacterium* sp. in the Remediation of Polycyclic Aromatic Hydrocarbon Wastes

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Abstract

Recent investigations in my laboratory on the biodegradation of PAHs has led to the isolation of a *Mycobacterium* sp., which was able to extensively degrade PAHs containing up to five fused aromatic rings. This microorganism has been shown to mineralize naphthalene (59.5%), phenanthrene (50.9%), pyrene (63.0%), fluoranthene (89.7%), 1-nitropyrene (12.3%), 6-nitrochrysene (2.0%), and 3-methylcholanthrene (1.6%). Interestingly, the 4-fused ring PAH, pyrene, was metabolized by the *Mycobacterium* sp. to both *cis*- and *trans*-4,5-dihydroxy-4,5-dihydropyrene. $^{18}O_2$ incorporation experiments showed that the formation of *cis*- and *trans*-dihydrodiol isomers were catalyzed by dioxygenase and monooxygenase enzymes, respectively. Similar studies with naphthalene indicated that the *Mycobacterium* initially hydroxylated naphthalene to form *cis*- and *trans*-1,2-dihydroxy-1,2-dihydronaphthalene in a ratio of 20:1, respectively. The *cis*-naphthalene dihydrodiol was further metabolized to ring cleavage products via the classical *meta* cleavage pathway. Initial oxidation of 1-nitropyrene occurred in the 4,5- and 9,10- positions to form *cis*-4,5- and 9,10-1-nitropyrene dihydrodiols. Fluorenone-1-carboxylic acid was identified as a predominant ring cleavage product in the degradation of fluoranthene by the *Mycobacterium*.

The ultimate usefulness of the *Mycobacterium* in the bioremediation of PAH contaminated sediments depends upon its survival and function in diverse ecosystems. The *Mycobacterium* survived and mineralized PAHs in sediment and water microcosms. Microcosms inoculated with the *Mycobacterium* showed enhanced mineralization, singly and as components in a mixture, for 2-methylnaphthalene, phenanthrene, pyrene and benzo[a]pyrene. Studies utilizing pyrene as a sole PAH substrate showed that the *Mycobacterium* survived in microcosms for six weeks in both the presence and absence of PAH exposure. The versatility of the PAH-degrading *Mycobacterium* and its potential for use in the bioremediation of PAH contaminated sediments will be discussed.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a major class of environmental contaminants originating from both petrogenic and pyrogenic sources (*22,24,25,27,28,34,38,41*). Many PAHs are cytotoxic, mutagenic and carcinogenic to both lower and higher eucaryotic organisms (*13,24,29,33,37*) (Figure 5.1.1). Due to their hydrophobic nature, most PAHs in aquatic ecosystems rapidly become associated with particles and are deposited in sediments. A variety of processes, including volatilization, sedimentation, chemical oxidation, photo-decomposition, and microbial degradation are important mechanisms for environmental loss of PAHs (Figure 5.1.2). Microbial degradation of PAHs can have a significant effect on the PAH distribution in sediment, especially near the sediment-water interface (*2,3,6,31*).

There is considerable interest in the use of microorganisms to decontaminated PAH-polluted environments (*42*). Successful bioremediation is dependent upon the availability of microorganisms which possess the catabolic enzymes needed to degrade PAHs. Mono- and dioxygenases are two groups of enzymes which are important to the microbial catabolism of PAHs. Dioxygenases incorporate both atoms of the oxygen molecule into the PAH. This dioxygenase reaction is the major mechanism for the initial oxidative attack on PAHs by bacteria, which leads to the formation of dihydrodiols that are in the *cis-* configuration (*6*). Enzymatic fission of the aromatic ring is also catalyzed by dioxygenases (Figure 5.1.3). In contrast to bacteria, fungi oxidize PAHs via a cytochrome P-450 monooxygenase by incorporating one atom of the oxygen molecule into the PAH and the other into water (*7-12*). Chemical pathways and enzymatic mechanisms for the microbial metabolism of PAHs containing two or three aromatic rings have been well studied (*6*). However, there are very few studies on the microbial degradation and detoxification of higher molecular weight PAHs. Our current knowledge on the microbial degradation of PAHs is summarized below:

1. Biodegradation of lower molecular weight PAHs by a wide variety of microorganisms has been demonstrated and the biochemical pathways have been investigated (*6*).

2. There is limited information on the microbial utilization of PAHs containing four or more aromatic rings; however, cometabolism of high molecular weight PAHs by bacteria has been demonstrated (*1,16,17,19,20,30,32,39,40*).

3. Biodegradation of unsubstituted PAHs always involves the incorporation of molecular oxygen catalyzed by monooxygenase(s) or dioxygenase(s) (*6*). However, there is also increasing interest and speculation concerning anaerobic decomposition of PAHs (*35,36*).

4. Many of the genes coding for bacterial degradation of PAHs are plasmid-associated (*5,45*).

5. Fungi hydroxylate PAHs as a prelude to detoxification, whereas bacteria oxidize PAHs as a prelude to ring fission and assimilation (*6,7-12*).

6. Fungal metabolism of PAHs is highly regio- and stereoselective (*8,11*).

7. White-rot fungi have the ability to cleave the aromatic rings of PAHs (*4*).

8. Microbial degradation of PAHs can occur under denitrifying conditions (*35,36*).

9. Lower molecular weight PAHs, such as naphthalene and phenanthrene, are degraded rapidly in sediments, whereas higher weight PAHs, such as benz[a]anthracene or benzo[a]pyrene, are quite resistant to microbial attack (*2,15,21*).

10. Environmental factors can have a significant effect on PAH biodegradation (*43*).

11. There are higher biodegradation rates for PAHs in PAH-contaminated sediments than in pristine sediments (*15,18,21*).

12. Procaryotic pathways for naphthalene metabolism predominate in sediments from freshwater and estuarine sediments (*18*).

Recent investigations in my laboratory on the biodegradation of PAHs has led to the isolation of a *Mycobacterium* sp. which is able to extensively degrade PAHs containing up to five fused aromatic rings (*16,19*). The ultimate usefulness of the *Mycobacterium* in the bioremediation of PAH-contaminated sediments depends upon its survival and function in diverse ecosystems (*17*). The versatility of the PAH-degrading *Mycobacterium* and its potential for use in the biodegradation of PAH contaminated sediments will be reported.

Materials and Methods:

Isolation of the polycyclic aromatic hydrocarbon degrading bacterium.

The bacterium was isolated from a 500 ml microcosm containing 20 g of sediment, 180 ml of estuarine water and 100 µg of pyrene (*16,19*). The sediment was obtained from a drainage pond chronically exposed to petrogenic chemicals. After incubation of the microcosm for 25 days under aerobic conditions, the sediment samples were serially diluted and screened for the presence of PAH degrading microorganisms (*16,19*).

The screening medium consisted of mineral salts medium (*44*) containing (per liter): NaCl, 0.3 g; $(NH_4)_2SO_4$, 0.6 g; KNO_3, 0.6 g; KH_2PO_4, 0.25 g; K_2HPO_4, 0.75 g; $MgSO_4 \cdot 7H_2O$, 0.15 g; LiCl, 20 µg; $CuSO_4 \cdot 5H_2O$, 80 µg; $ZnSO_4 \cdot 7H_2O$, 100 µg; $Al_2(SO_4)_3 \cdot 16H_2O$, 100 µg; $NiCl \cdot 6H_2O$, 100 µg; $CoSO_4 \cdot 7H_2O$, 100 µg; KBr, 30 µg; KI, 30 µg; $MnCl_2 \cdot 4H_2O$, 600 µg; $SnCl_2 \cdot 2H_2O$, 40 µg; $FeSO_4 \cdot 7H_2O$, 300 µg; agar, 20 g and distilled H_2O, 1000 ml.

The surfaces of the agar plates were sprayed with a 2% (wt/vol) solution of a PAH dissolved in acetone: hexane (1:1, vol/vol) and dried overnight at 35°C to volatilize the carrier solvents. This treatment resulted in a visible and uniform surface coat of the PAH on the agar. Inocula (100 µl) from the 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions of microcosm sediments were gently spread with sterile glass rods onto the agar surface; the plates were inverted and incubated for three weeks at 24°C in sealed plastic bags to conserve moisture.

When colonies surrounded by clear zones (Figure 5.1.4) due to polycyclic aromatic hydrocarbon uptake and utilization were observed (after 2 to 3 weeks), they were subcultured into fresh mineral salts medium containing 250 µg/l each of peptone, yeast extract, and soluble starch and 0.5 µg/ml of a PAH dissolved in dimethylformamide. After three successive transfers, a bacterium was isolated which was able to degrade pyrene, a PAH containing 4 aromatic rings.

Growth of Organism and Culture Conditions.

The *Mycobacterium* sp. was grown in 125 ml Erlenmeyer flasks containing 30 ml of basal salts medium (*19*) supplemented with 250 µg/ml each of peptone, yeast extract, and soluble starch and 0.5 µg/ml of pyrene dissolved in dimethylformamide. The cultures were incubated in the dark at 24°C for 72 h on a rotary shaker operating at 150 rpm. Cells in the mid-logarithmic phase of growth were harvested by centrifugation at 8000 x *g* for 20 min at 4°C. The harvested cells were resuspended in sterile 0.1 M *tris*(hydroxymethyl)aminomethane buffer (pH 7.5) at a concentration of 3 x 10^6 cells/ml and used as inoculum for studies of PAH biodegradation.

Biodegradation experiments.

Biodegradation of PAHs by the *Mycobacterium* sp. was monitored in a flow-through microcosm test system (*14,23,26*). This system enables simultaneous monitoring of mineralization (complete degradation to CO_2) and the recovery of volatile metabolites, nonvolatile metabolites, and residual PAH. Microcosms in this test system consisted of 500 ml glass mini-tanks containing 100 ml of minimal basal salts medium, 0.92 µCi of ^{14}C-labeled PAH and 50 µg of unlabeled PAH. The PAHs used and their sources were [1,4,5,8-^{14}C]naphthalene (5.10 mCi/mmole), Amersham/Searle Corp., Arlington Heights, Ill.; [9-^{14}C]phenanthrene (19.3 mCi/mmole), Amersham/Searle; [3-^{14}C]fluoranthene (54.8 mCi/mmole), Chemsyn Science Laboratories, Lenexa, Kansas; [4-^{14}C]pyrene (30.0 mCi/mmole), Midwest Research Institute, Kansas City, Mo.; 3-[6-^{14}C]-methylcholanthrene (13.4 mCi/mmole) New England Nuclear Corp., Boston, MA. and 6-nitro-[5,6,11,12-^{14}C]chrysene (57.4 mCi/mmole), Chemsyn Science Laboratories.

Each microcosm was inoculated with 1.5 x 10^4 cells/ml, mixed twice weekly, incubated at 24° C for 14 days, and continuously purged with compressed air. The gaseous effluent from each microcosm was directed through a volatile-organic-trapping column containing 7 cm of polyurethane foam and 500 mg of Tenax GC (Alltech Associates, Inc., Deerfield, Il.) and a $^{14}CO_2$ trapping column (50 ml of monoethanolamine: ethylene glycol, 7:3, vol/vol). Mineralization was measured at various intervals by adding duplicate 1 ml aliquots from the $^{14}CO_2$ trapping column to scintillation vials containing 15 ml of a 1:1 mixture of Fluoralloy and methanol (Beckman Instruments Co., Fullerton, Ca.). Autoclaved inoculated microcosms, and microcosms lacking the *Mycobacterium* sp., were included to detect abiotic PAH degradation.

Results and Discussion

There are four major objectives in my research program concerning PAH biodegradation.

1. To determine the relationships between chemical structure and PAH degradation by measuring mineralization rates in microcosms, getting good mass balance accountability of undegraded PAH and of volatile and non-volatile metabolites.
2. To isolate microorganisms from environmental sites chronically exposed to PAHs, which have the ability to degrade PAHs containing four or more aromatic rings.
3. To elucidate biochemical pathways and reaction mechanisms for PAH degradation in environmental samples.
4. To determine if PAH-degrading bacteria would be useful in the biological decontamination and detoxification of PAH-polluted sites.

It is clear from previous investigations that it is relatively easy to isolate microorganisms, using classical enrichment and plating techniques, which can utilize lower molecular weight PAHs containing 2 or 3 rings. The focus of research in my laboratory is to isolate microorganisms which degrade the higher molecular weight PAHs. A summary of our recent investigations is reported below.

Enrichment of PAH degrading bacterium.

A pyrene-degrading bacterium was isolated by direct enrichment from sediment samples taken from an oil field near Port Aransas, Texas (Figure 5.1.4). By repeated streaking and isolation, we obtained an isolate, strain Pyr-1, which was identified as a *Mycobacterium* sp. on the basis of the following morphological and biochemical properties (*44*). It formed gram-positive, acid-fast rods (1.4 µm in length and 0.7 µm in width). The 15 biochemical tests, mole percent G+C analysis of 66% and the characterization of the mycolic acids with a carbon chain length of C_{58} to C_{64} were consistent with the assignment of this organism to the genus *Mycobacterium*.

Utilization of PAHS by Mycobacterium.

The *Mycobacterium* utilized naphthalene, phenanthrene, fluoranthene, pyrene, 3-methylcholanthrene, 1-nitropyrene and 6-nitrochrysene when grown in mineral salts medium supplemented with low levels of peptone, yeast extract and soluble starch (*16*). This bacterium was unable to utilize these PAHS as the sole source of carbon and energy. Pyrene induced *Mycobacterium* cultures readily degraded naphthalene (59.5%), phenanthrene (50.9%), fluoranthene (89.7%), pyrene (63.0%), 1-nitropyrene (12.3%), 3-methylcholanthrene (1.6%), and 6-nitrochrysene (2.0%) to CO_2 within 48 h of incubation (Figure 5.1.5). Pathways for the initial degradation of pyrene, naphthalene, fluoranthene, and 1-nitropyrene are shown in Figures 5.1.6-5.1.9.

The *Mycobacterium* sp. initially oxidized pyrene to form both pyrene *cis*- and *trans*-4,5-dihydrodiols (*20*). Oxygen-18 incorporation experiments showed that both atoms of the *cis*-pyrene dihydrodiol were derived from molecular oxygen where as only one atom of molecular oxygen was incorporated into the *trans*-pyrene dihydrodiol (Figure 5.1.6). 4-Phenanthroic acid, 4-hydroxyperinaphthenone, cinnamic acid were identified as ring fission products (*20*). The *Mycobacterium* sp. initially oxidized naphthalene in the 1,2-positions to form naphthalene-1,2-dihydrodiols. Similar to pyrene oxidation both the naphthalene *cis* and *trans*-1,2-dihydrodiols were isolated in a ratio of 20:1. The naphthalene *cis*-1,2-dihydrodiols is further metabolized to salicylate and catechol by the classical bacterial oxidation of naphthalene pathway (Figure 5.1.7). The *Mycobacterium* sp. extensively degrades fluoranthene to CO_2 (Figure 5.1.8). However, a ring cleavage metabolite was isolated and identified as

9-fluorenone-1-carboxylic acid. 1-Nitropyrene is degraded very slowly by the *Mycobacterium* sp. and little mineralization occurs which indicates that the nitro-substituent may sterically block initial enzymatic attack and ring cleavage enzymes since pyrene is rapidly degraded. However, 1-nitropyrene *cis*-4,5- and 9,10-dihydrodiols were isolated and characterized (Figure 5.1.9).

Microcosm studies to evaluate the PAH-degrading capacity and survival of the Mycobacterium when added to pristine sediments.

Figure 5.1.10 indicates that 2-methylnaphthalene and phenanthrene were mineralized to 10% and 14%, respectively after 28 days in microcosms containing sediment and water from De Gray Reservoir, Arkadelphia, Arkansas. De Gray Reservoir is a pristine lake, which receives relatively little chemical inputs, and has a low-PAH degrading microbial population (*15,17,18*). When similar microcosms were inoculated with the *Mycobacterium* sp. (1.5×10^5 cells/g of moist sediment), mineralization of 2-methylnaphthalene and phenanthrene increased to 26% and 71%, respectively. In addition, pyrene and benzo[a]pyrene degradation were observed, whereas previously we did not see degradation of high-molecular weight PAHs in De Gray Reservoir sediments lacking the *Mycobacterium*. Therefore, the *Mycobacterium* sp. competed with indigenous microflora and enhanced mineralization of PAHs (*17*).

Our research indicates that the *Mycobacterium* sp. isolated from an oil-contaminated estuarine site is very versatile and can mineralize low and high molecular weight PAHs. The process is co-oxidation, since low levels of organic nutrients are necessary to initiate growth and metabolism of the PAHs. The mechanism of oxidation is unique, since the *Mycobacterium* has both mono- and dioxygenases to catalyze the initial attack on the PAH.

In conclusion, when one discusses the use of microorganisms in the remediation of hazardous wastes, such as PAHs, some bioremediation issues that should be addressed are:

(1) A complete understanding of the chemical and ecological characterization of the site.

(2) More data on the fate, metabolism and kinetics of high-molecular weight PAH biodegradation at the site.

(3) Biochemistry and mechanisms of many of the high-molecular weight PAH degradative pathways.

(4) What conditions will insure the survival of the biological detoxification system?

(5) How can the biological detoxification system be effectively transported to the site?

(6) Development of procedures for employing immobilized cells to decontaminate PAH contaminated soils.

(7) Is bioremediation a cost effective means of cleanup of PAH contaminated wastes?

(8) How do you get the PAH degrading microorganisms (large biomass) there and make them grow and function?

(9) What is the fate of plasmid DNA or recombinant strains in wastewater or sediments?

(10) How do we optimize a PAH degrading microbial system for environmental use?

(11) Basic research on coupling aerobic and anaerobic biodegradation systems.

(12) Research on specific bacteria used at a site, such as salt tolerant or chemical tolerant bacteria.

References

1. Barnsley, E.A. (1975). The bacterial degradation of fluoranthene and benzo[a]pyrene. *Can. J. Microbiol.*, **21:** 1004-1008.

2. Bauer, J.E., and D.G. Capone (1985). Degradation and mineralization of the polycyclic aromatic hydrocarbons anthracene and naphthalene in intertidal marine sediments. *Appl. Environ. Microbiol.*, **50:** 81-90.

3. Bauer, J.E., and D.G. Capone (1988). Effects of co-occurring aromatic hydrocarbons on the degradation of individual polycyclic aromatic hydrocarbons in marine sediment slurries. *Appl. Environ. Microbiol.*, **54:** 1649-1655.

4. Bumpus, J.A. (1989). Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium. Appl. Environ. Microbiol.*, **55:** 154-158.

5. Burlage, R.S., S.W. Hooper and G.S. Sayler (1989). The TOL (pWWO) catabolic plasmid. *Appl. Environ. Microbiol.*, **55:** 1323-1328.

6. Cerniglia, C.E. and M.A. Heitkamp (1989). Microbial degradation of polycyclic aromatic hydrocarbons in the aquatic environment. **In:** *Metabolism of polycyclic aromatic hydrocarbons in the aquatic environment*, U. Varanasi (ed.), CRC Press Inc., Boca Raton, FL.

7. Cerniglia, C.E., W.L. Campbell, J.P. Freeman, and F.E. Evans (1989). Identification of a novel metabolite in phenanthrene metabolism by the fungus *Cunninghamella elegans. Appl. Environ. Microbiol.*, **55:** 2275-2279.

8. Cerniglia, C.E., W.L. Campbell, P.P. Fu, J.P. Freeman, and F.E. Evans (1990). Stereoselective fungal metabolism of methylated anthracenes. *Appl. Environ. Microbiol.*, **56:** 661-668.

9. Cerniglia, C.E., J.P. Freeman, G.L. White, R.F. Heflich, and D.W. Miller (1985). Fungal metabolism and detoxification of the nitropolycyclic aromatic hydrocarbon 1-nitropyrene. *Appl. Environ. Microbiol.*, **50:** 649-655.

10. Cerniglia, C.E., D.W. Kelly, J.P. Freeman, and D.W. Miller (1986). Microbial metabolism of pyrene. *Chem. Biol. Interact.*, **57:** 203-216.

11. Cerniglia, C.E., D.W. Miller, S.K. Yang, and J.P. Freeman (1984). Effects of fluoro substituents on the fungal metabolism of 1-fluoronaphthalene. *Appl. Environ. Microbiol.*, **48:** 294-300.

12. Cerniglia, C.E., G.L. White, and R.H. Heflich (1985). Fungal metabolism and detoxification of polycyclic aromatic hydrocarbons. *Arch. Microbiol.*, **50:** 649-655.

13. Dipple, A., R.C. Moschel, and C.A.H. Bigger (1984). Polynuclear aromatic carcinogens. **In:** *Chemical carcinogens. 2nd ed.*, C.E. Searle (ed.), American Chemical Society, Washington, D.C., pp. 41-163.

14. Heitkamp, M.A. and C.E. Cerniglia (1986). Microbial degradation of *t*-butylphenyl diphenyl phosphate: A comparative microcosm study among five diverse ecosystems. *Toxicity Assessment,* **1:** 103-122.

15. Heitkamp, M.A. and C.E. Cerniglia (1987). Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. *Environ. Toxicol. Chem.*, **6:** 535-546.

16. Heitkamp, M.A. and C.E. Cerniglia (1988). Mineralization of polycyclic aromatic hydrocarbons by a bacterium isolated from sediment below an oil field. *Appl. Environ. Microbiol.*, **54:** 1612-1614.

17. Heitkamp, M.A. and C.E. Cerniglia (1989). Polycyclic aromatic hydrocarbon degradation by a *Mycobacterium* sp. in microcosms containing sediment and water from a pristine ecosystem. *Appl. Environ. Microbiol.*, **55:** 1968-1973.

18. Heitkamp, M.A., J.P. Freeman, and C.E. Cerniglia (1987). Naphthalene biodegradation in environmental microcosms: estimates of degradation rates and characterization of metabolites. *Appl. Environ. Microbiol.*, **53:** 129-136.

19. Heitkamp, M.A., W. Franklin and C.E. Cerniglia (1988). Microbial metabolism of polycyclic aromatic hydrocarbons: Isolation and characterization of a pyrene degrading bacterium. *Appl. Environ. Microbiol.*, **54:** 2549-2555.

20. Heitkamp, M.A., J.P. Freeman, D.W. Miller and C.E. Cerniglia (1988). Pyrene degradation by a *Mycobacterium* sp.: Identification of ring oxidation and ring fission products. *Appl. Environ. Microbiol.*, **54:** 2556-2565.

21. Herbes, S.E., and L.R. Schwall (1978). Microbial transformation of polycyclic aromatic hydrocarbons in pristine and petroleum-contaminated sediments. *Appl. Environ. Microbiol.*, **35:** 306-316.

22. Hites, R.A., R.E. Laflamme and J.G. Windsor (1980). Polycyclic aromatic hydrocarbons in marine/aquatic sediments: Their ubiquity,. **In:** *Petroleum in the marine environment.* L. Petrakis and F.T. Weiss (eds.), Advances in Chemistry Series, American Chemical Society, Washington, D.C., pp. 289-311.

23. Huckins, J.N., J.D. Petty and M.A. Heitkamp (1984). Modular containers for microcosm and process model studies on the fate and effects of aquatic contaminants. *Chemosphere*, **13:** 1329-1341.

24. International Agency for Research on Cancer (1983). Polynuclear Aromatic Compounds. Part 1, Chemical, environmental and experimental data. **In:** *IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans*, World Health Organization, Lyon, France, pp. 95-451.

25. Jacob, J., W. Karcher, J.J. Belliardo and P.J. Wagstaffe (1986). Polycyclic aromatic hydrocarbons of environmental and occupational importance. *Fresenius Z. Anal. Chem.*, **323:** 1-10.

26. Johnson, B.T., M.A. Heitkamp and J.R. Jones (1984). Environmental and chemical factors influencing the biodegradation of phthalic acid esters in freshwater sediments. *Environ. Pollut. Ser. B*, **8:** 101-118.

27. Johnson, A.C. and D. Larsen (1985). The distribution of polycyclic aromatic hydrocarbons in the surficial sediments of Penobscot Bay (Maine, USA) in relation to possible sources and to other sites worldwide. *Mar. Environ. Res.*, **15:** 1-16.

28. Jones, K.C., J.A. Stratford, K.S. Waterhouse, and N.B. Vogt (1989). Organic contaminants in Welsh soils: polynuclear aromatic hydrocarbons. *Environ. Sci. Technol.*, **23:** 540-550.

29. Keith, L.H., and W.A. Telliard (1979). Priority pollutants I. a perspective view. *Environ. Sci. Technol.*, **13:** 416-423.

30. Kelley, I., and C.E. Cerniglia (1990). The metabolism of fluoranthene by a species of *Mycobacterium*. *J. Ind. Microbiol.* (in press).

31. Lewis, D.L., R.E. Hodson and L.F. Freeman (1984). Effects of microbial community interactions on transformation rates of xenobiotic chemicals. *Appl. Environ. Microbiol.*, **48:** 561-565.

32. Mahaffey, W.R., D.T. Gibson and C.E. Cerniglia (1988). Bacterial oxidation of chemical carcinogens: Formation of polycyclic aromatic acids from benz[a]anthracene. *Appl. Environ. Microbiol.*, **54:** 2415-2423.

33. Martelmans, K.S. Haworth, T. Lawlor, W. Speck, B. Tainer and E. Zeiger (1986). Salmonella mutagenicity tests II. Results from the testing of 270 chemicals. *Environ. Mutagen.*, **8 (Suppl. 7):** 1-119.

34. Means, J.C., S.G. Ward. J.J. Hassett and W.L. Banwart (1980). Sorption of polynuclear aromatic hydrocarbons by sediments and soils. *Environ. Sci. Technol.*, **14:** 1524-1528.

35. Mihelcic, J.R. and R.G. Luthy (1988). Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. *Appl. Environ. Microbiol.*, **54:** 1182-1187.

36. Mihelcic, J.R. and R.G. Luthy (1988). Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. *Appl. Environ. Microbiol.*, **54:** 1188-1198.

37. Miller, E.C. and J.A. Miller (1981). Searches for ultimate chemical carcinogens and their reactions with cellular macromolecules. *Cancer*, **47:** 2327-2345.

38. Morehead, N.R., B.J. Eadie, B. Lake, P.D. Landrum and D. Berner (1986). The sorption of PAH onto dissolved organic matter in Lake Michigan waters. *Chemosphere*, **15:** 403-412.

39. Mueller, J.G., P.J. Chapman, B.O. Blattmann, and P.H. Pritchard (1990). Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. *Appl. Environ. Microbiol.*, **56:** 1079-1086.

40. Mueller, J.G., P.J. Chapman, P.H. Pritchard (1989). Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. *Appl. Environ. Microbiol.*, **55:** 3085-3090.

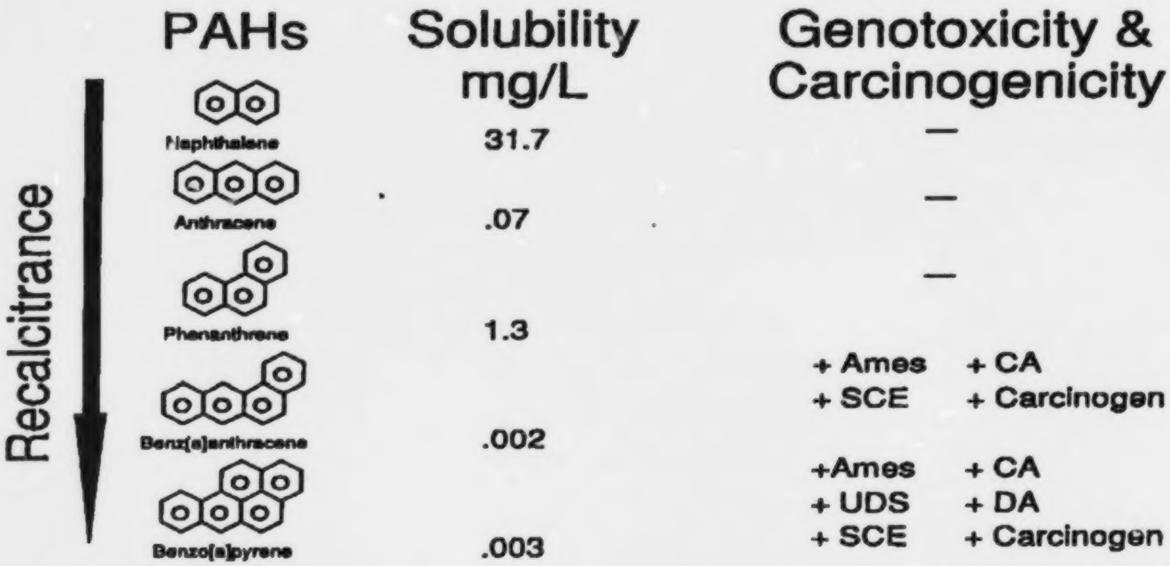
41. National Academy of Sciences (1983). Polycyclic aromatic hydrocarbons: Evaluation of sources and effects. National Academy Press, Washington, D.C.

42. Nicholas, R.B. (1987). Biotechnology in hazardous waste disposal: An unfulfilled promise. *ASM News*, **53:** 138-142.

43. Shiaris, M.P. (1989). Seasonal biotransformation of naphthalene, phenanthrene and benzo[a]pyrene in surficial estuarine sediments. *Appl. Environ. Microbiol.*, **55:** 1391-1399.

44. Skerman, V.B.D. (1967). A guide to the identification of the genera of bacteria, 2nd ed. The Williams & Wilkins Co., Baltimore.

45. Williams, P.A. (1981). Genetics of biodegradation. In: *Microbial degradation of xenobiotics and recalcitrant compounds*, T. Leisinger, R. Hutter, A.M. Cook, and J. Nuesch (eds.), Academic Press, Inc., New York, pp. 97-130.


PAHs
Solubility mg/L
Genotoxicity & Carcinogenicity
Recalcitrance
Naphthalene
31.7
—
Anthracene
.07
—
Phenanthrene
1.3
—
Benz[a]anthracene
.002
+ Ames
+ CA
+ SCE
+ Carcinogen
Benzo[a]pyrene
.003
+Ames
+ CA
+ UDS
+ DA
+ SCE
+ Carcinogen

DA = DNA adducts
CA = chromosomal abberations
Ames = Salmonella typhimurium reversion assay
SCE = sister chromated exchange
UDS = unscheduled DNA synthesis

Figure 5.1.1. The structures and chemical and toxicological characteristics of polycyclic aromatic hydrocarbons.

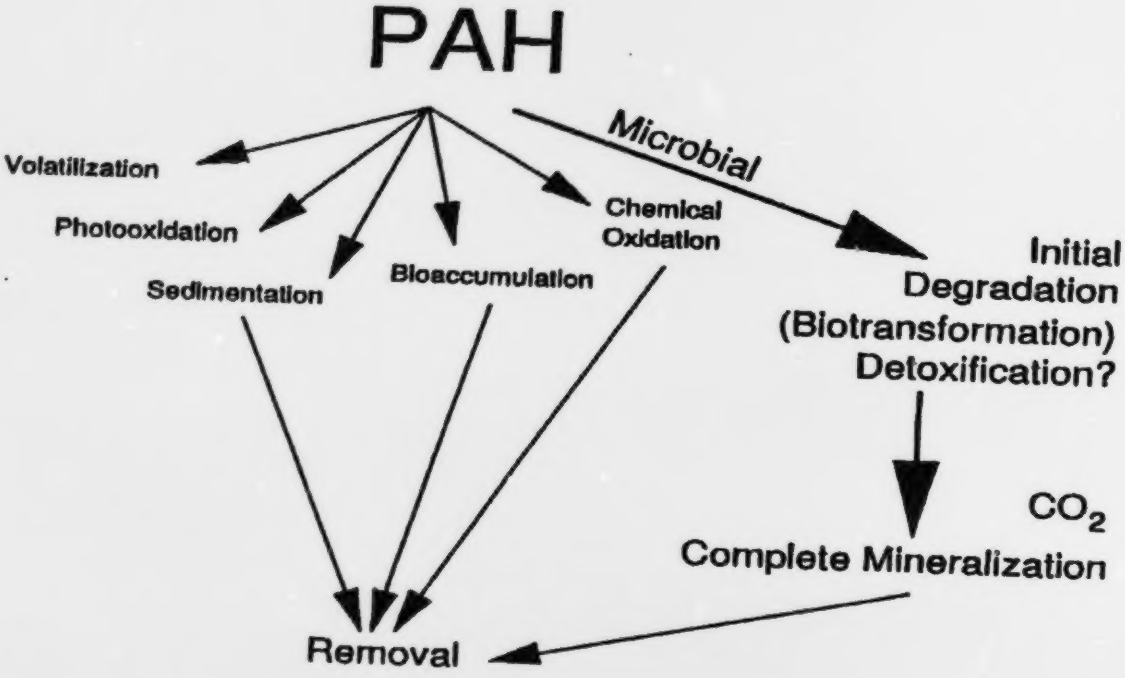

PAH
Volatilization
Photooxidation
Sedimentation
Bioaccumulation
Chemical Oxidation
Microbial
Initial Degradation (Biotransformation) Detoxification?
CO2
Complete Mineralization
Removal

Figure 5.1.2. Schematic representation of the environmental fate of polycyclic aromatic hydrocarbons.

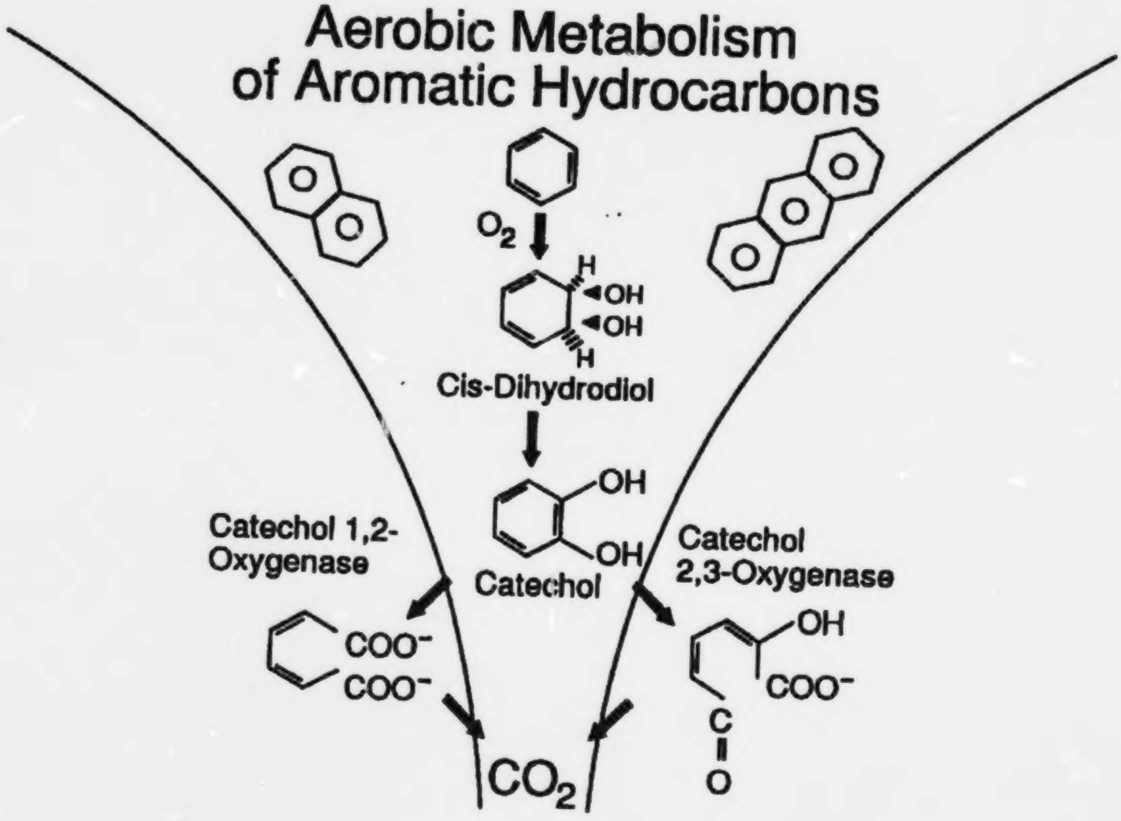

Aerobic Metabolism of Aromatic Hydrocarbons
O2
H
OH
OH
H
Cis-Dihydrodiol
OH
OH
Catechol
Catechol 1,2-Oxygenase
Catechol 2,3-Oxygenase
COO⁻
COO⁻
OH
COO⁻
C
O
CO2

Figure 5.1.3. Major pathways of bacterial oxidation of polycyclic aromatic hydrocarbons.

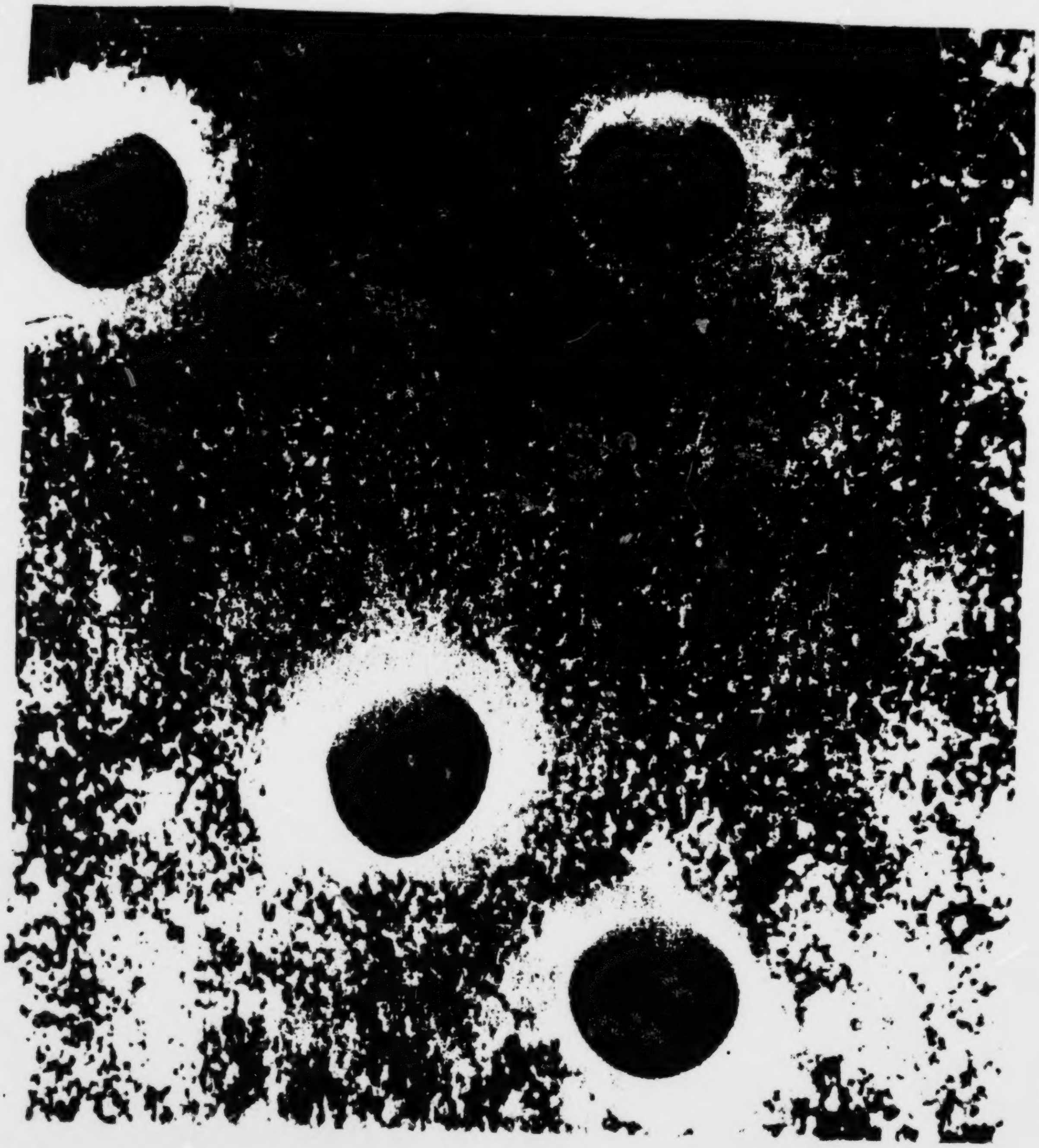


Figure 5.1.4. Photograph of *Mycobacterium* sp. colonies on MBS agar containing low-levels of nutrients and coated with pyrene. The clear zones around the bacterial colonies indicate pyrene utilization.

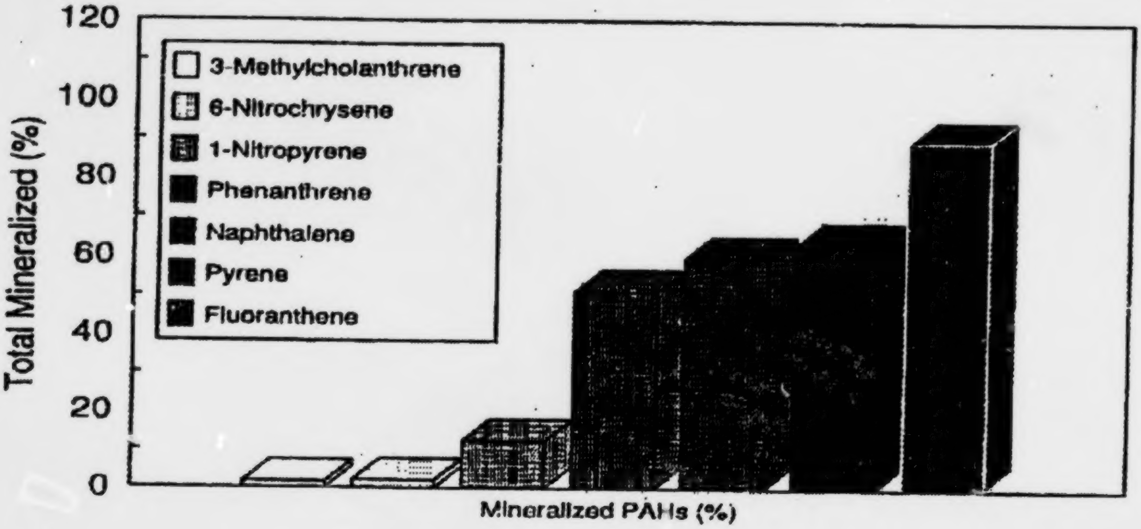


Figure 5.1.5. Mineralization of naphthalene, phenanthrene, pyrene, fluoranthene, 1-nitropyrene, 6-nitrochrysene, and 3-methylcholanthrene by the *Mycobacterium* sp.

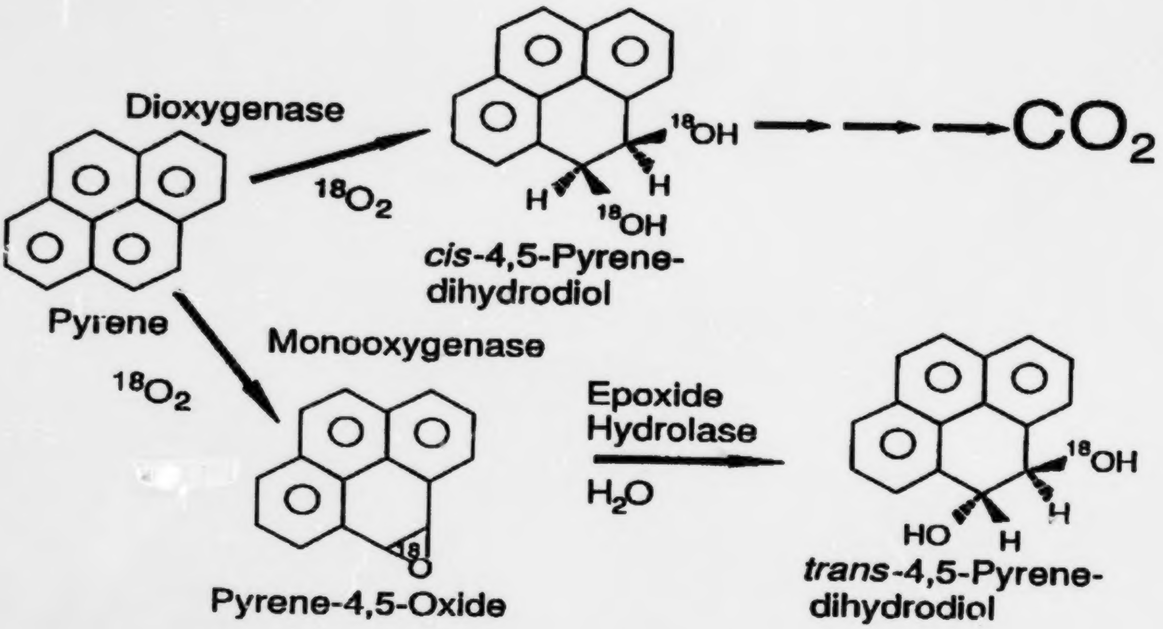

Dioxygenase
$^{18}O_2$
^{18}OH
H
H
^{18}OH
cis-4,5-Pyrene-dihydrodiol
CO_2
Pyrene
Monooxygenase
$^{18}O_2$
Epoxide Hydrolase
H_2O
18
O
Pyrene-4,5-Oxide
^{18}OH
H
HO
H
trans-4,5-Pyrene-dihydrodiol

Figure 5.1.6. The pathways utilized by the *Mycobacterium* sp. for the oxidation of pyrene.

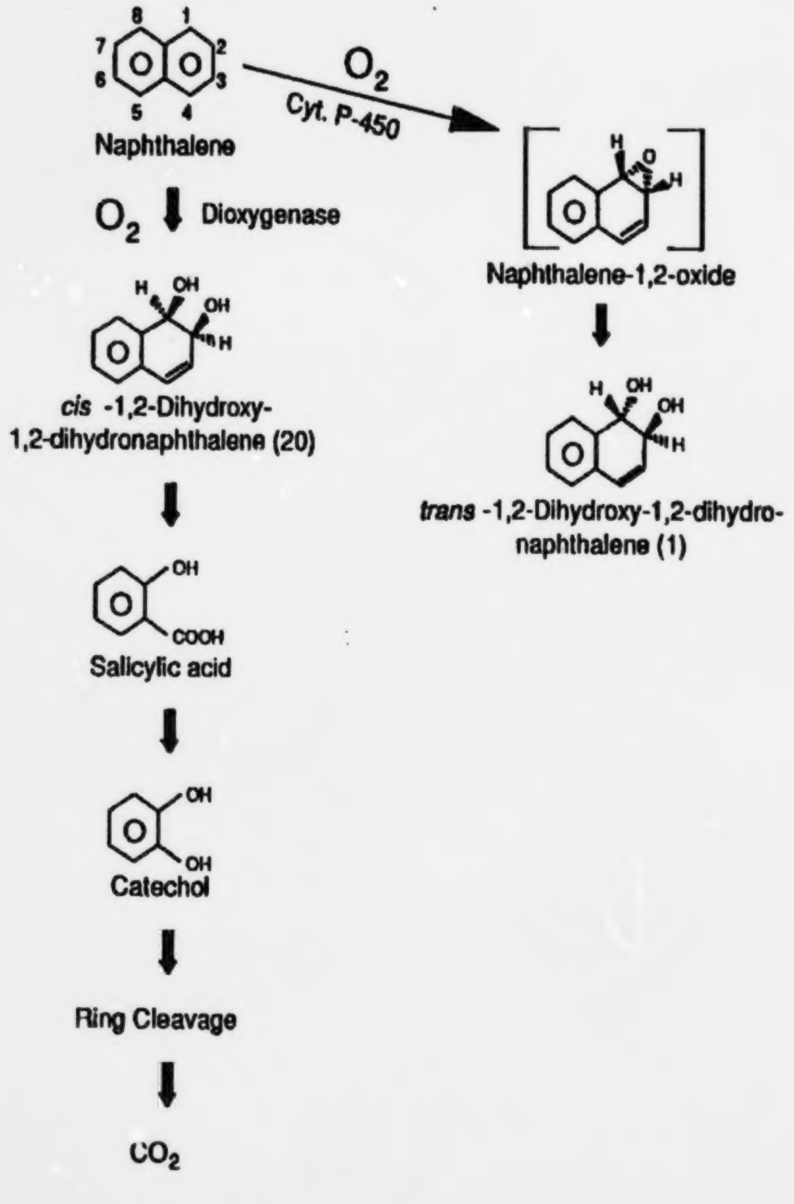

8
1
7
2
6
3
5
4
Naphthalene
O_2
Cyt. P-450
H
O
H
Naphthalene-1,2-oxide
H
OH
OH
H
trans -1,2-Dihydroxy-1,2-dihydro-naphthalene (1)
O_2
Dioxygenase
H
OH
OH
H
cis -1,2-Dihydroxy-1,2-dihydronaphthalene (20)
OH
COOH
Salicylic acid
OH
OH
Catechol
Ring Cleavage
CO_2

Figure 5.1.7. The pathways utilized by the *Mycobacterium* sp. for the oxidation of naphthalene.

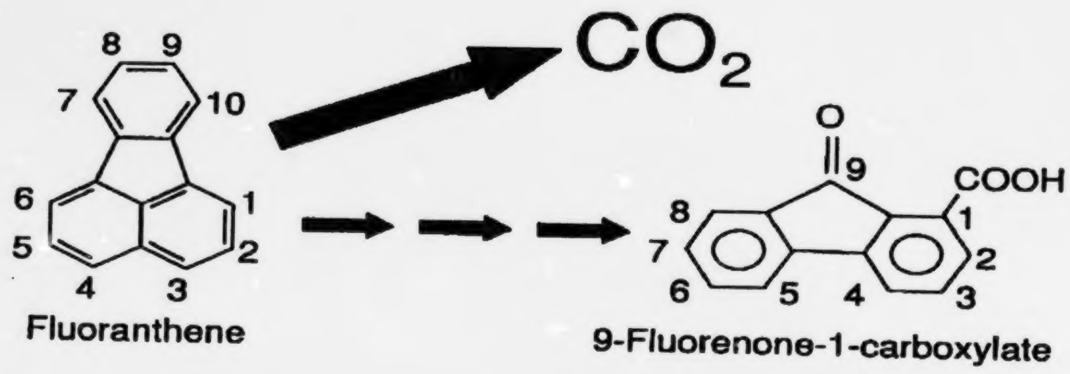

CO2
Fluoranthene
9-Fluorenone-1-carboxylate
COOH
1
2
3
4
5
6
7
8
9
10

Figure 5.1.8. The pathways utilized by the *Mycobacterium* sp. for the oxidation of fluoranthene.

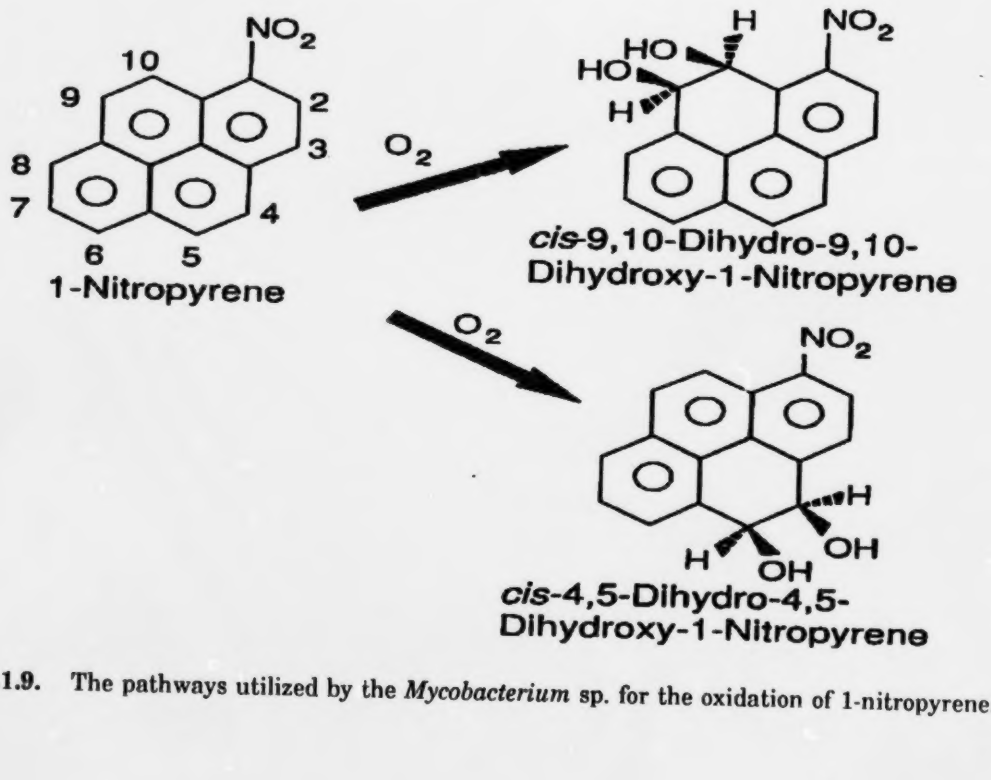

1-Nitropyrene
NO2
O2
O2
cis-9,10-Dihydro-9,10-Dihydroxy-1-Nitropyrene
cis-4,5-Dihydro-4,5-Dihydroxy-1-Nitropyrene
HO
HO
H
OH

Figure 5.1.9. The pathways utilized by the *Mycobacterium* sp. for the oxidation of 1-nitropyrene.

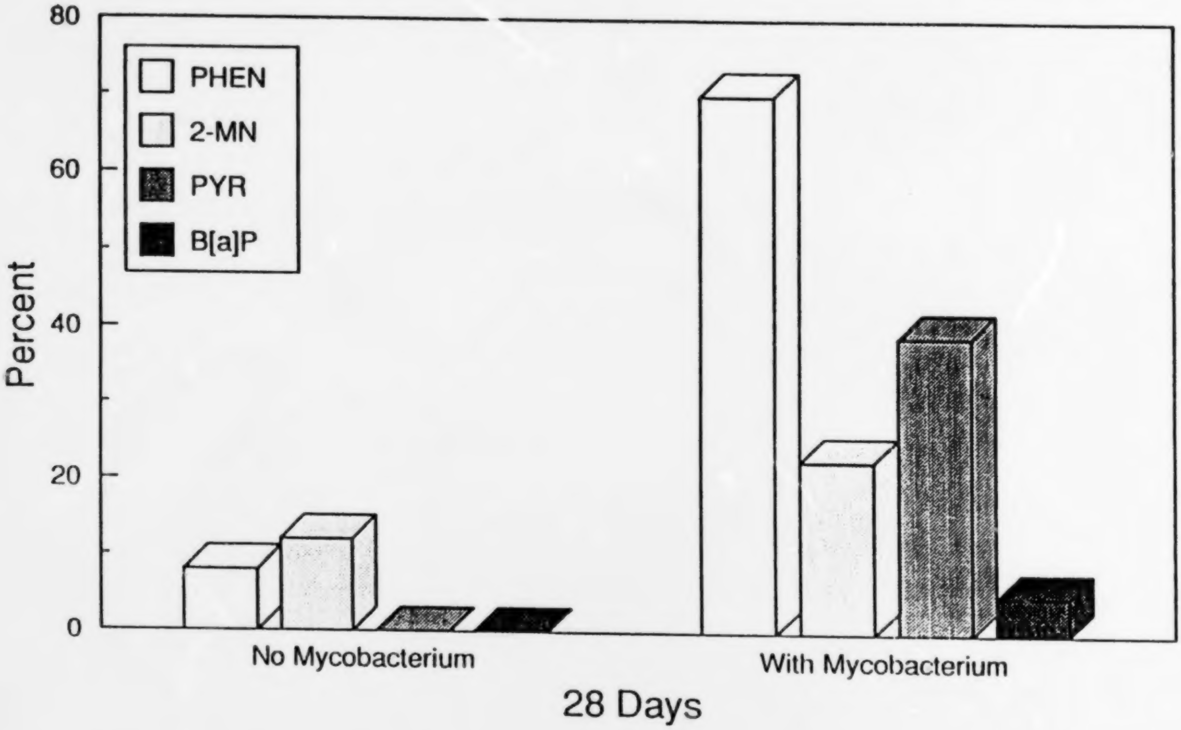


Figure 5.1.10. Mineralization of phenanthrene, 2-methylnaphthalene, pyrene, and benzo[a]pyrene in microcosms from De Gray Reservoir sediments and water with and without *Mycobacterium* inoculation.

5.2 Fungal Degradation of Hazardous Wastes

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Detoxification of hazardous wastes is important as a means to reduce the risk associated with such waste. The potential of biological processes to detoxify hazardous waste is beginning to be recognized. The ability to degrade/detoxify organic and inorganic waste constituents requires two complementary features: microbial competence to degrade target pollutants and effective contact between the biomass and the pollutants. The competence of an organism is best understood in terms of the biochemicals (enzymes) that enable the organism to convert the contaminant chemical to a non-toxic end product. Due to the variety of possible chemicals forming pollutant mixtures, either a single organism of exceptional competence or a multiplicity of compatible organisms of complimentary competence is necessary.

The bioavailability or contact between the organism and the pollutant substrate is a function of mass transfer and the most appropriate reactor conditions that maintain the activity of the selected degrading organisms. Selection and development of reactor configurations and operating conditions are necessary to sustain economic operation of the treatment.

An example of a single organism of high competence is the wood degrading fungus, *Phanerochaete chrysosporium*. This fungus possesses great potential to degrade aromatic components of toxic and hazardous waste, based on the widely recognized ability to degrade lignin, a persistent biogenic polymer. The degradation of lignin required largely non-specific enzyme systems to accomplish this remarkable biodegradation. The fungus is also non-pathogenic to plants and animals permitting possible application of this technology to solve a variety of environmental contamination problems. The related development of the fungal biomass as a food supplement for livestock serves to underscore the potential utility and benign aspects associated with this organism.

The current catalog of pollutants degraded by this fungus ranges from polynuclear aromatic hydrocarbons, polychlorinated biphenyls, pesticides to dyes. The wide range ability of this organism to degrade these diverse pollutant classes is a tribute to the activity of the enzymes systems secreted.

Two areas of liquid and soil treatment have been investigated recently. The liquid treatment shows promise and is under pilot scale evaluation. However, the application to soil contamination has shown the most exciting success in the last year.

The ability of the organism to treat under field conditions has recently been evaluated. An Oshkosh, Wisconsin site was selected for field trial applications of the white rot fungal treatment to contaminated soil. The area of application was a former "tank farm" where above-ground storage tanks contained a wood preservative formulation known as "Woodlife". The composition of this product was predominantly mineral spirits (high boiling pentanes and hexanes) and 5% pentachlorophenol. Extensive screening for pentachlorophenol in the tank farm identified concentrations of 1 to 4435 mg/kg to depths of 30 cm. Within the confines of a protective berm for the tank farm, the field trial study was laid out according to a specific treatment design. After thorough mixing of the soil, nine plot borders were installed in a three-by-three configuration. The plot borders were constructed deep. Plot borders were worked into the soil surface and filled to a depth of 25 cm with soil outside the border. Approximately 370 kg (dry weight) of soil were added to each plot. Two different fungi (*P. chrysosporium* and *P. sodida*) were selected as candidate treatment species. In each case, the fungi were added to the contaminated area through the use of inoculated wood chips with the appropriate fungal species. The treatability trial began in early August 1989 and continues to the end of September 1989. The pentachlorophenol concentration was depleted by 82% and 85% respectively, for the two fungal species after 46 days of treatment.

The investigation of field utility of this organism will continue to be pursued. The scope of the fungal treatment is not limited to the currently selected series of pollutants under study. Additional pollutant classes such as PCBs, pesticides and herbicides will be explored under field conditions to determine the general utility of this organism. Development of the best reactor configuration for field use and maintenance of the organism's biodegrading activities is underway.

5.3 Recent Studies on the Microbial Degradation of PAHs and Their Relevance to Bioremediation

James G. Mueller[1], Peter J. Chapman[2],
Suzanne E. Lantz[1] and P. Hap Pritchard[2]
(presented by John E. Rogers[3])

Southern BioProducts, Inc., Gulf Breeze, Florida[1],
U.S. EPA Environmental Research Laboratories at
Gulf Breeze, Florida[2] and Athens, Georgia[3]

Polycyclic aromatic hydrocarbons (PAHs) are an ubiquitous class of chemicals whose presence in the environment can be attributed to a number of natural and anthropogenic sources (*13*). While the majority of these chemicals are innocuous, several, especially the higher-molecular-weight (HMW) PAHs, have been shown to exhibit adverse health effects. A number of areas contaminated with this class of chemical (i.e., coal gasification sites, petroleum refineries, creosote waste sites) often contain sufficient amounts of HMW PAH carcinogens and other toxic chemicals to pose a significant threat to environmental and human health.

Biological degradation represents a major route through which PAHs and many other organic chemicals are removed from contaminated environments. By and large, lower-molecular-weight PAHs containing 2 or 3 rings are readily degraded biologically (*1,5*), and the catabolic pathways for the degradation of these compounds by certain organisms have been established (*2,3,4*). Conversely, HMW PAHs are less readily biodegraded and do persist in contaminated environments. Consequently much less is known of the microbiology and biochemistry of their degradation. This dearth of information is of particular concern since HMW PAHs represent the greatest risk to public and environmental health.

Because HMW PAHs are less amenable to microbial attack, their removal from contaminated environments has proven to be especially difficult for bioremediation technologies. However, for bioremediation to be considered as an acceptable remedial action alternative for these types of wastes, biotreatment processes must prove to be capable of destroying these chemicals in a reliable, timely and predictable manner. To this end, efforts were undertaken to isolate microorganisms capable of degrading HMW PAHs. These studies resulted in the discovery of the first axenic bacterial cultures which utilized HMW PAHs as sole sources of carbon and energy for growth (*6,7*). Moreover, complete mineralization of a number of these compounds has been demonstrated (*8*).

Making use of this new source of novel biocatalysts, a multi-phasic biological treatment strategy has been developed which effectively integrates physical separation technology (membrane extraction) with microbial degradation processes. Recent bench-scale studies have evaluated the effectiveness of a tri-phasic treatment approach (Figure 5.3.1) for remediation of creosote-contaminated soil and sediment present at the American Creosote Works Superfund site at Pensacola, Florida: soil washing (phase 1), membrane extraction/pollutant fractionation (phase 2) and biodegradation (phase 3). A bi-phasic approach comprising membrane extraction followed by biodegradation of concentrated organics was also evaluated. Performance data from these studies clearly demonstrate the superiority of the multi-phasic biotreatment strategy over conventional biotreatment approaches such as land-farming, slurry-phase and *in situ* bioremediation (*9,10,11,12*).

REFERENCES

1. Bossert, I. and R. Bartha (1986). Structure-biodegradability relationships of polycyclic aromatic hydrocarbons in soil. *Bull. Environ. Contamin. Toxicol.*, **37:** 490-495.

2. Cerniglia, C.E. (1984). Microbial metabolism of polycyclic aromatic hydrocarbons. *Adv. Appl. Microbiol.*, **30:** 31-71.

3. Cerniglia, C.E. and M.A. Heitkamp (1989). Microbial degradation of polycyclic aromatic hydrocarbons (PAH) in the aquatic environment. **In:** *Metabolism of PAH in the Aquatic Environment*, U. Varanasi (ed.), CRC Press, Boca Raton, Fl. pp. 41-68

4. Gibson, D.T. and V. Subramanian (1984). Microbial degradation of aromatic hydrocarbons. **In:** *Microbial Degradation of Organic Compounds*, D. Gibson (ed.), Marcel Dekker, Inc. New York, pp. 181-252.

5. McGinnis, G.D. , H. Borajani, L.K. McFarland, D.F. Pope, D.A. Strobel and J.E. Mathews (1988). Characterization and laboratory soil treatability studies for creosote and pentachlorophenol sludges and contaminated soil. EPA 600/2-88/055.

6. Mueller, J.G., P.J. Chapman and P.H. Pritchard (1989). Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. *Appl. Environ. Microbiol.*, **55:** 3085-3090.

7. Mueller, J.G., P.J. Chapman, Beat O. Blattmann and P.H. Pritchard (1990). Isolation and characterization of a fluoranthene-utilizing strain of *Pseudomonas paucimobilis*. *Appl. Environ. Microbiol.*, **56:** 1079-1086.

8. Mueller, J.G., P.J. Chapman, S.E. Lantz, B.O. Blattmann, and P.H. Pritchard (1990). Mineralization of fluoranthene by *Pseudomonas paucimobilis* strain EPA505 and identification of biotransformation products. *Appl. Environ. Microbiol.*, (submitted).

9. Mueller, J.G., S.E. Lantz, B.O. Blattmann and P.J. Chapman (1990). Bench-scale evaluation of alternative biological treatment processes for the remediation of creosote contaminated materials: solid-phase bioremediation. *Environ. Sci. Technol.*, (submitted).

10. Mueller, J.G., S.E. Lantz, B.O. Blattmann and P.J. Chapman (1990). Bench-scale evaluation of alternative biological treatment processes for the remediation of creosote-contaminated materials: slurry-phase bioremediation. *Environ. Sci. Technol.*, (submitted).

11. Mueller, J.G., D.P. Middaugh, S.E. Lantz, and P.J. Chapman (1990). Biodegradation of creosote and PCB in contaminated groundwater: chemical and biological assessment. *Appl. Environ. Microbiol.* (submitted).

12. Middaugh, D.E., J.G. Mueller, R.L. Thomas, S.E. Lantz, M.J. Hemmer, G.T. Brooks and P.J. Chapman (1990). Detoxification of creosote-contaminated groundwater by ultrafiltration: chemical and biological assessment. *Arch. Environ. Contam. Toxicol.* (submitted).

13. National Academy of Sciences (1983). Polycyclic aromatic hydrocarbons: Evaluation of sources and effects. National Academy Press, Washington, D.C.

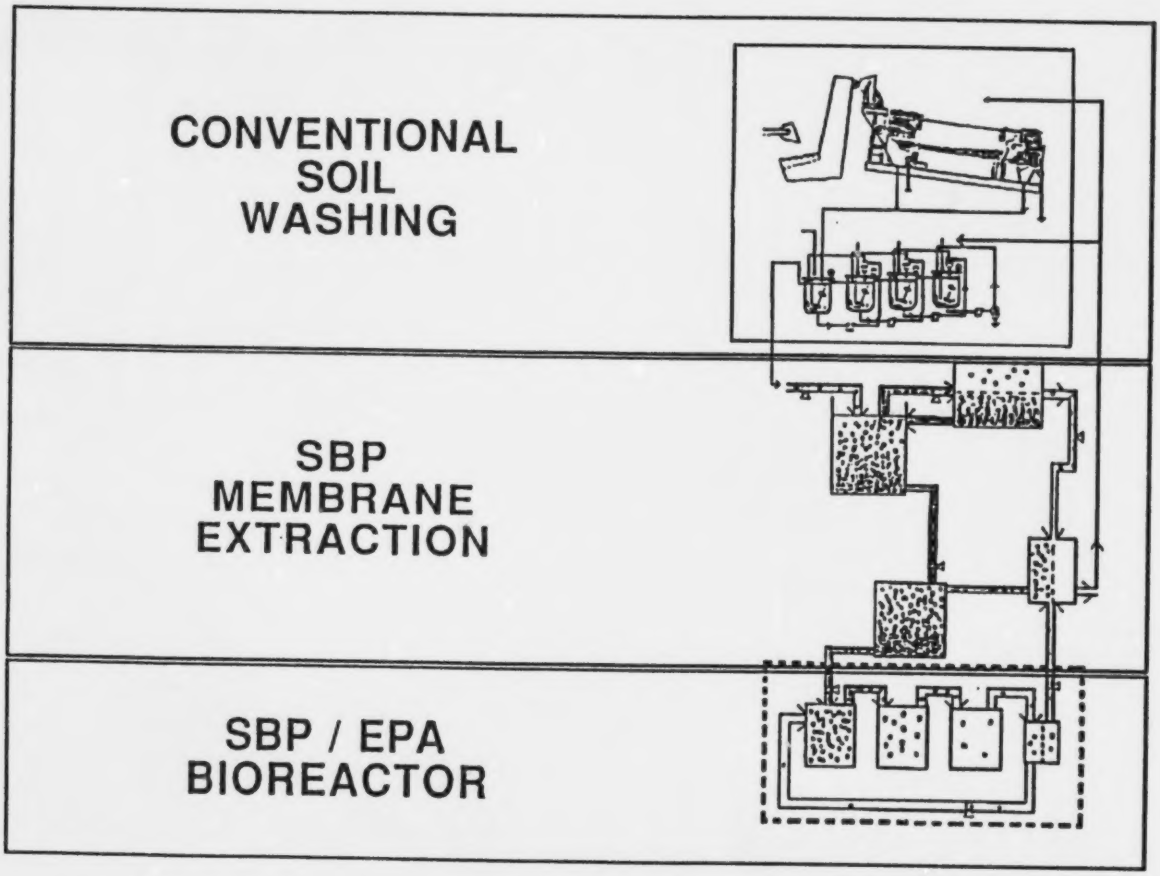
CONVENTIONAL SOIL WASHING
SBP MEMBRANE EXTRACTION
SBP / EPA BIOREACTOR

Figure 5.3.1. Tri-phasic treatment approach

5.4 Biological Remediation of Contaminated Sediments in the Netherlands

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T.N.O., Apeldoorn
The Netherlands

INTRODUCTION

In the Netherlands, contaminated sediments are manifest as an environmental problem in a dual way:

- As contaminated aquatic soil with the corresponding environmental impact
- As a dredged-sludge problem because many watercourses in the Netherlands must be dredged for nautical and for water management reasons.

The dredged-sludge problem is currently dominating. This means that remediation of contaminated sediments in the Netherlands refers to dredged-sludge remediation in particular. Until a few years ago, all dredged sludges were increasingly being processed in order to improve their quality. In this respect, a number of harbors had been remediated that were seriously contaminated with PAH, oil, and metals, specifically. Their remediation included the dredging and processing of the sludge by means of classification and dewatering into a fraction for beneficial use and into a concentrate to be disposed.

TNO is one of the institutes that carries out research to improve the remediation technology. This research into the processing of dredged sludge takes place within a program in the order of approximately $2 million (U.S.) for 1990. Also participating in the research project are government, trade, and private companies.

The outlines of the program are as follows:

- Optimization of environmental dredging. The purpose is to remove contaminated sediments selectively.
- Classification of dredged sludges into fractions with different contaminant concentrations.
- Biological, chemical, and physical treatment of the sludge aimed at immobilization of the contamination.

This paper gives a survey of the current state of full-scale aquatic soil remediation in the Netherlands and the development of biological remediation of dredged sludges.

CURRENT STATE OF CONTAMINATED SEDIMENT REMEDIATION IN PRACTICE

Since 1985, technology has been applied to restrict the quantitative volume of contaminated dredged sludge to be disposed of. The process applied, consists of a combination of two techniques: hydrocyclones and dewatering. In this way a relatively clean fraction is separated from the dredged sludge while the residual fraction is reduced in volume as much as possible.

Hydrocyclones

Particle classification is carried out by hydrocyclones (Figure 5.4.1). A hydrocyclone has one inlet, two outlets, the vortex finder, and the apex nozzle. The outlet flows are called overflow and underflow. The fluid feed enters the cyclone tangentially bringing about a downward flow that circulates near the wall of the cyclone. The flow reverses near the apex into an upstream in the center of the cyclone and leaves the cyclone by way of the vortex finder.

When a heavy particle enters the feed, the downward flow moves this particle by centrifugal force to the wall of the cyclone. The particle leaves the cyclone through the apex. A less heavy particle does not have enough time to reach the wall of the cyclone; thus, it leaves the cyclone together with the larger part of the water in the overflow. In this way, a hydrocyclone classifies dredged sludge into heavy sand particles, on the one hand, and into fines and organic material, on the other hand.

Fines and organic material have a high contaminant content compared with sand, on account of the differences in sorption properties. This means that hydrocyclones separate a relatively clean sand fraction from the slime fraction in which a concentration of contaminants is found.

The effect of hydrocyclones is characterized by two aspects: the distribution of the dry matter [E_{dm}][1] and the distribution of the contaminant [E_s][2].

The applicability of hydrocyclones for the treatment of contaminated dredged sludge was recognized as early as 1983. The technique has been applied in a number of dredging operations, but does not always offer a solution, in particular, not for dredged sediments with a high content of very small particles and high organic matter content (peat). Figure 5.4.2 shows a number of results obtained in hydrocyclone experiments with dredged sludge from various sites, as well as with various contaminants. The effect of hydrocyclones is more favorable as the data point is closer to the origin of the diagram. From the figure it appears that hydrocyclones often give good results, but not always.

Dewatering

There is various dewatering equipment. Three apparatuses qualify for the dewatering of dredged sludges and of the slime fraction of dredged sludges: the belt press, the filter press, and the decanter. In general, it can be said that the filter press results in the highest dry-matter content, whereas the decanter results in the lowest dry-matter content. The use of flocculants is, in most cases, necessary for dewatering. When a belt press and filter press are applied, flocculants bring about a good filterability; in the case of a decanter, flocculants help in reaching a clear decantate. All three apparatuses mentioned are applicable to practical dredged sludge treatment.

The purpose of dewatering is to reach a volume reduction of the sludge or slime fraction produced by hydrocyclones. Figure 5.4.3 shows the effect of dewatering on volume, starting from a slime fraction with a dry-matter content of 5% after using hydrocyclones. The figure shows that as the dry-matter content increases, a considerable volume reduction is reached in the first instance. However, at higher dry-matter contents (approximately 40%), the volume decreases less strongly at increased dry-matter contents.

Since dewatering is aimed at volume reduction, it appears from this figure that further dewatering becomes less cost effective. Dewatering costs increase strongly as a higher dry-matter contents are reached.

---

[1] E_{dm} = separation efficiency for the dry matter; this is the percentage of the dry matter that leaves the hydrocyclone as underflow (sand fraction)

[2] E_s = separation efficiency for the contaminants; this is the percentage of the contaminants that leave the hydrocyclone with the underflow

Cases

Table 5.4.1 gives the results of a number of practical cases of the treatment of dredged sludges by hydrocycloning/dewatering. It appears that, on a practical scale, favorable results have been reached by using hydrocyclones and dewatering.

In a number of remediation cases that are currently being carried out, the following bottlenecks have been ascertained:

- It turns out that the information obtained in the preliminary investigation strongly differs from the real situation. For instance, the composition of the soil strongly deviates from the composition expected on the basis of the preliminary investigation. This makes it difficult for the contractor in charge of the remediation to meet the results described in his quotation.

- After using hydrocyclones, the sand fraction, in some cases, still shows a high PAH concentration because the PAHs are not adsorbed to the slime fraction, but are present as some kind of tar particle that can hardly be separated from the sand.

Future research will pay considerable attention to these two bottlenecks.

BIOLOGICAL REMEDIATION

General

Dutch research into biological remediation is particularly focused on the biodegradation of oils and PAHs because these organic micropollutants occur most frequently. TNO has carried out laboratory-scale exploratory research into the biological remediation of the dredged sludges contaminated with mineral oils and PAHs (Table 5.4.2). This research has shown that effective biological cleaning is possible for a number of dredged sludges. Spontaneous degradations have been found in these dredged sludges, if the conditions for these sludges are biologically favorable (as in the case in a bioreactor). From a biological point of view, such a degradation often goes by quickly.

Present research is done along two lines:

1. Development of biological remediation techniques up to a practical scale. This concerns the development of designs for the biodegradation process that link up with the dredging process.

2. Broadening of the fundamental knowledge pertaining to the degradation of PAHs and other substances such as chlorinated hydrocarbons.

At present, Dutch research emphasizes the former line.

For the practical application of biological remediation TNO has three treatment ways in view:

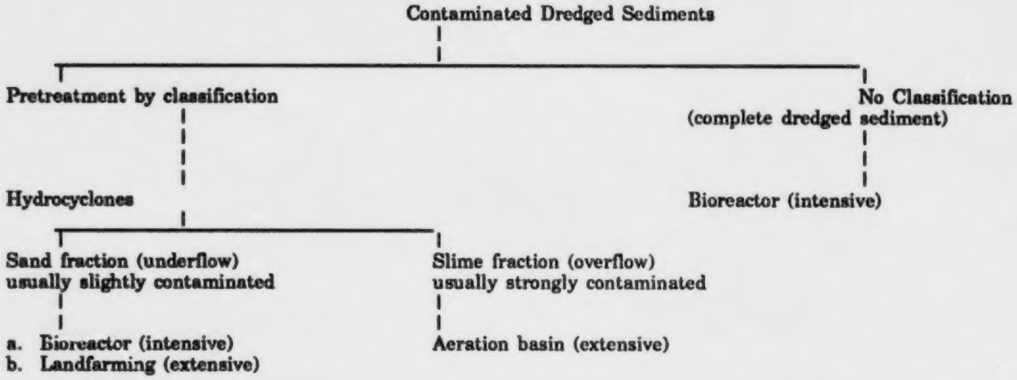
1. Large scale, extensive treatment in aeration basins

2. Intensive treatment in bioreactors

3. Landfarming

These three ways of treatment, together, form a complete process for remediation contaminated dredged sediments. The differences in dredged sediments refer to: granular composition, distribution of the contaminants among the particle size fractions, contaminant content, and degradation rate.

COMPLETE DIAGRAM FOR THE BIOLOGICAL REMEDIATION
OF CONTAMINATED DREDGED SEDIMENTS


Contaminated Dredged Sediments
Pretreatment by classification
No Classification
(complete dredged sediment)
Hydrocyclones
Bioreactor (intensive)
Sand fraction (underflow)
usually slightly contaminated
Slime fraction (overflow)
usually strongly contaminated
a. Bioreactor (intensive)
b. Landfarming (extensive)
Aeration basin (extensive)

This diagram is based on the following major arguments:

- *No Classification*

  Due to its physical behavior, the non-separated (i.e., "complete") sediment can be treated in a bioreactor only. It often contains too many fine particles for landfarming, in other words, its porosity is too small. For an aeration basin there are too many coarse particles which can hardly be brought into suspension.

- *Pretreatment by classification*

  - The most important reason for classification is that it results in two fractions which can be treated separately very well; whereas, this is not true for the original dredged sludge.
  - Depending on contaminant content, the (usually) slightly contaminated fraction can have a direct beneficial use or can be remediated by way of bioreactors or landfarming. The advantage is that part of the dredged sediment can be treated in a relatively short time. Landfarming demands a coarse particle size due to the high porosity needed. The sand fraction has these characteristics.
  - The slime fraction can be treated as a liquid; consequently, it can be remediated as waste water. Therefore, an aeration basin is a large-scale possibility.

Apart from the above aspects pertaining to the composition of the dredged sediment which determines the treatment method to be applied, factors of a pragmatic character and local conditions play an important part in the treatment method to be chosen, such as:

- *Available space.*

  → If there is sufficient space available, an aeration basin can be considered. Such an aeration basin demands a large surface area; depending on the quantity of dredged sediment to be treated, this may approximate some tens of thousands m^2. If there is not sufficient space available, the bioreactor offers a possibility (up to a maximum of some hundreds m^2).

- *Available time.*

  → If the remediation should take place within a short period of time (and if the degradation rate is sufficiently high), bioreactor treatment is the appropriate method.

  → If the remediation may take a relatively long period of time (from months up to one or two years), there are possibilities for large-scale, extensive methods (landfarming, aeration basin).

- *Quantity of dredged sediment to be remediated.*

  → If the remediation involves a relatively small quantity of dredged sediment, a bioreactor can be used.

  → If the remediation involves a considerable quantity, it is necessary to apply a large scale method.

The biological treatment methods (bioreactor, aeration basin, and landfarming) mentioned above are all subject to investigation.

Intensive Versus Extensive Treatment Methods

Practical biodegradation offers a choice between intensive and extensive methods.

Intensive implementation methods

An intensive implementation method is aimed at:

- operating a process as intensively as possible (with much exertion)
- thus realizing conditions as optimum and verifiable as possible
- resulting in as short as possible a treatment period.

These implementation methods refer to process-type treatment methods (e.g. bioreactor)

Extensive implementation methods

These implementation methods are meant to:

- operate remediation methods with relatively slight exertion (extensive)
- usually implying less optimum and verifiable conditions.

These implementation methods refer to large-scale, more or less batchwise treatments, like biodegradation by means of landfarming and/or treatment as a slurry in an aeration basin.

Whether an intensive or an extensive way of implementation is chosen for remedial operations, it is determined by a number of choice criteria. Below, by means of some important choice criteria more detailed grounds are given as to why, in some cases, the application of large-scale, extensive ways of implementation can be an alternative for intensive (process-type) methods.

- *Period of time*

The period in which a certain quantity of dredged sediment can be cleaned depends on the capacity of the remediation method applied. For extensive methods the relevant quantity of dredged sediment to be cleaned is treated in one batch during a long time. Intensive methods of implementation may involve short treatment times, but remediation plants have a relatively limited capacity. In fact, practice will show that the remedial operation for a reasonably sized quantity of dredged sediment also takes a long time by way of intensive methods. This means that the application of intensive implementation methods does not necessarily have much advantage over extensive methods, as far as period of time is concerned.

- *Costs*

In view of the simplicity of extensive implementation techniques and the small exertion needed, it is expected that these techniques will cost less than intensive implementation techniques. An important condition in this respect is that the costs of building a facility in which the large-scale remediation can take place (e.g. depot or basin) cannot be fully taken into account in the remediation costs, because:

- the depot/basin has to be built anyway to store the dredged sediment; in that case, the depot/basin will not be built just for the remedial operation
- The depot/basin can be used various times to remediate dredged sediments from different sites.

With respect to the construction of an aeration basin, in practice it is possible for such a basin to be part of the harbor that is screened off from the rest. In that case, the costs are expected to be considerably lower than for a new basin.

- *Space needed*

Much space is needed for extensive implementation methods, in contrast to intensive methods. If this space is available, extensive implementation methods are a reasonable alternative.

- *Implementation in dredging operations*

In the Netherlands the remediation of dredged sludge is carried out by dredging companies and contractors. Past experience has demonstrated that implementation of new technology leads to great problems within companies. Extensive implementation methods are more compatible with the factory management.

Figure 5.4.4 gives the results of laboratory experiments carried out with respect to the biodegradation of PAHs in a Rotterdam harbor sediment (i.e., 'Geulhaven' sediment). The following three treatments were carried out:

1. Treatment of the original sample in a bioreactor. The laboratory bioreactor is a rotating drum with baffles, with a contents of approximately 10 liters.
2. After hydrocyclones, the slime fraction of the 'Geulhaven' sample was treated in an aeration column; the material was aerated four times a day for one hour, with a total quantity of 25 m^3 air/m^3 suspension per day. This is approximately 10% of the air quantity fed into a biological sewage treatment.
3. Treatment of the sand fraction in a laboratory landfarm. A 20 cm thick layer of sand fraction was put into a 1 m^2 tray. About once a month the sand was mixed with a hand shovel. To prevent the sand from drying out, it was moistened every week resulting in a dry matter content of approximately 80-90%.

From Figure 5.4.4, it is quite evident that intensive bioreactor treatment goes by quickly. After a longer period, however, high degradation percentages are also reached by way of extensive methods. The scale for these laboratory experiments is 10 m^3.

TNO's Concept for Extensive Bioremediation of Dredged Sludge

Based on results, some of which are mentioned in this paper, a plan has been developed for the extensive treatment of dredged sludges. This plan comprises the separation of the dredged sludge by hydrocyclones, after which, the sand is treated in a landfarm, and slime in an aeration basin.

Landfarming of the sand fraction

Landfarming is a technique that is frequently applied in practical (terrestrial) soil remediation. Much experience has been gained with respect to the degradation of mineral oils and PAHs in particular. Briefly, the contaminated soil is put down in layers (20 - 50 cm thick) in a field that is especially equipped for this purpose. Care is usually taken for:

- Manuring
- Good water balance (draining or watering)
- Oxygen supply by means of tillage (plowing, harrowing, working with a rotary cultivator)
- Increasing the porosity-increasing means (such as peat and bark) for a better oxygen and water balance.

Sometimes,

- Inoculation with special cultivation or activated sludge takes place, as well as
- Temperature increase by means of leading steam or hot water through pipes, or constructing a covering of transparent plastic foil (greenhouse).

Treatment times depend on contaminant content and vary from six months to two years.

Aeration basin for the treatment of the slime fraction

The size of the basin is determined by the quantity of suspension to be treated and the treatment period. The quantity of suspension (overflow of the hydrocyclone) depends on the quantity of dredged sediment to be cleaned; usually a minimum of 1,000 m^3 suspension per site is assumed. From research it can be deduced that the treatment period for this extensive method will be at least some months. This means that basins of some thousands to some tens of thousands m^3 are needed. In this respect one should think of:

- (temporary) depots that are dug or surrounded by earthen dikes
- screened off part of the relevant site.

For the aeration of basins up to a size of ten thousand m^3, the distribution of air within the basin is an important aspect. In this respect, a comparison is made with an aeration basin of a sewage treatment plant, where as short as possible a treatment time is strived after. This means:

a. Installation of aeration elements across the whole surface of the aeration space, and
b. A sufficient mixing of the waste water (turbulence).

These two conditions are not considered feasible for aeration basins that have to treat the slime fraction, in view of the size of such basins. Taking care that there is sufficient turbulence and oxygen for the aeration basin is considered non-realistic. The TNO plan considers the installation of a large-scale treatment depot for the slime fraction, with intermittent aeration. This aeration is realized by moving a pontoon with aerators and mixers slowly to and fro across the length of the basin.

FINAL REMARKS

Further research into biological remediation will incorporate the following:

- Together with trade and industry, further auxiliary research into the scale-up of the techniques presented in this paper

- Further fundamental research into biodegradation. This aspect will be considered by both TNO and universities.

In the Netherlands, the introduction of treatment technology for contaminated sediments in dredging operations has started only recently. The introduction of relatively simple techniques, such as hydrocyclonage, already appears to cause many problems. These problems are among other things the result of :

- An inadequate preliminary survey of the site to be dredged; in this way remediation plans are based on incomplete information which later turns out to be incorrect.
- The dredging companies underestimating the degree of complexity of the remediation technology.
- The research results being scaled up too quickly to a practical scale, researchers underestimate the implementation problems.

In the Netherlands, there is still a relatively large antitreatment lobby; it consists of representatives of government and companies who do not consider the treatment of dredged sludge worthwhile and want to dump everything. This lobby has intensified as a result of the introductory problems. Therefore, it is of utmost importance to start with simple technology for the development of remediation technology for contaminated sediments; only at a later stage is a more sophisticated technology desirable. This is one of the most important reasons why we are convinced of the feasibility of the TNO concept for biological remediation of contaminated sediments.

Table 5.4.1. Results of Practical Hydrocyclone Applications

Project	Barendrecht 1985	Roozendaal 1986	Nijerkerk 1986	Dordrecht 1988
Process (*)	1	2	3	4
Capacity (m^3/h)	20	18	300	300
Separation	20	50-60	50-60	50-60
Contaminants diameter (micron)	metals oils	metals, oils	PAH	PAH, metals
E_{dm}	50%	20%	70%	60%
E_s	metals: ± 15%	metals: 1-5% oils: ± 0.5%	PAH: 5-10%	PAH: ± 5% oils: ± 10%
Concentration in sand (mg/kg)	Zn: 169 Cu: 28 Cd: 1.8	Zn: 63 Cu: 18 Cd: 0.2 oils: 93	PAH: 1-2.9	PAH: 0.38 Zn: 150 Cu: 38 Cd: 0.9
Volume reduction by hydrocyclones/ dewatering	-	75	50	-

* 1. Test installation consisting of a storage basin, a preseparator (CBC = Circulation Bed Classifier), a buffer basin and hydrocyclones.
2. Installation consisting of a hydrocyclone and a sieve belt press.
3. Installation consisting of a sieve, three hydrocyclones, a sediment tank and a sludge depot. Flocculants have been dosed in the delivery pipe to the depot for a quick first sedimentation, thus making a quick water drainage possible.
4. See 3. The sludge depot has been replaced by a flat-bottom craft in which the fine fraction has settled.

Table 5.4.2. Results of Biodegradation for Various Sediment Samples

PAH content mg/kg*	Geulhaven	Scheveningen	Dordrecht	Dodewaard	Amstel/Drecht
Original material	212	351	817	156	372
After 7 days	49	237	217	150	320
After 30 days	22	188	232	-	333
After 60 days	16	175	145	125	275
Oil Content mg/kg					
Original material	12000	2580	2826	379	1372
After 7 days	-	-	-	-	1045
After 30 days	1040	1027	768	357	-
Cleaning efficiency (%)					
PAH after 60 days	92	50	82	20	26
Oil after 7 days	-	-	-	-	24
Oil after 30 days	91	60	73	6	-

* 16 PAH (EPA)

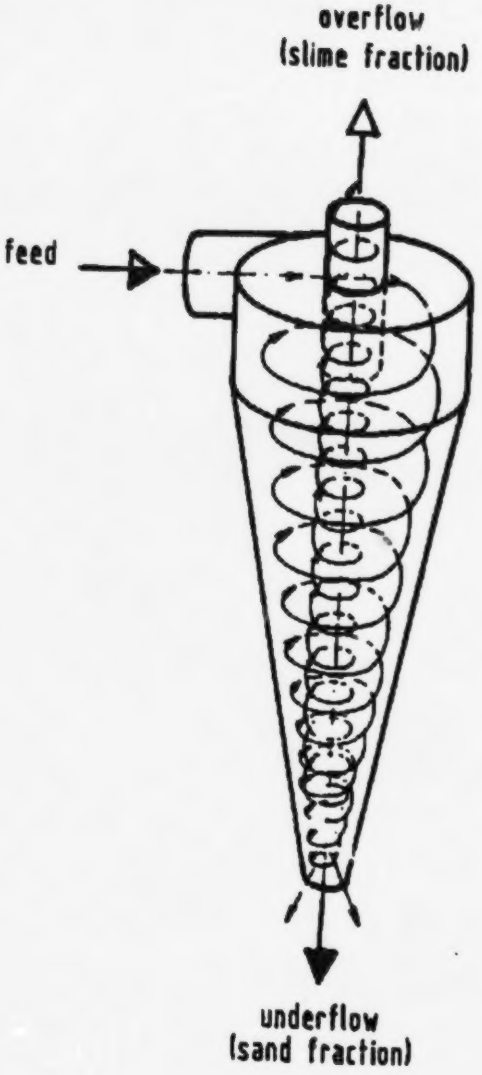

overflow
(slime fraction)
feed
underflow
(sand fraction)

Figure 5.4.1. Hydrocyclone

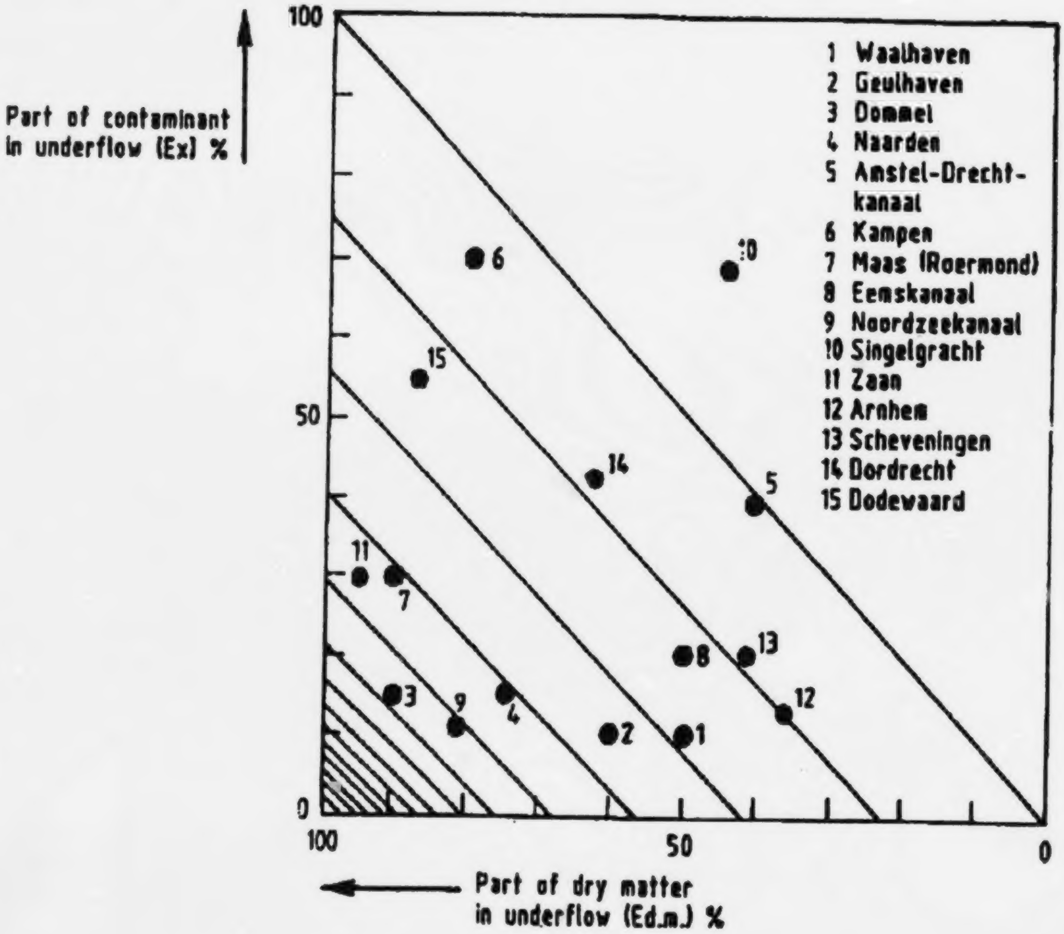


Figure 5.4.2. Hydrocyclone results.

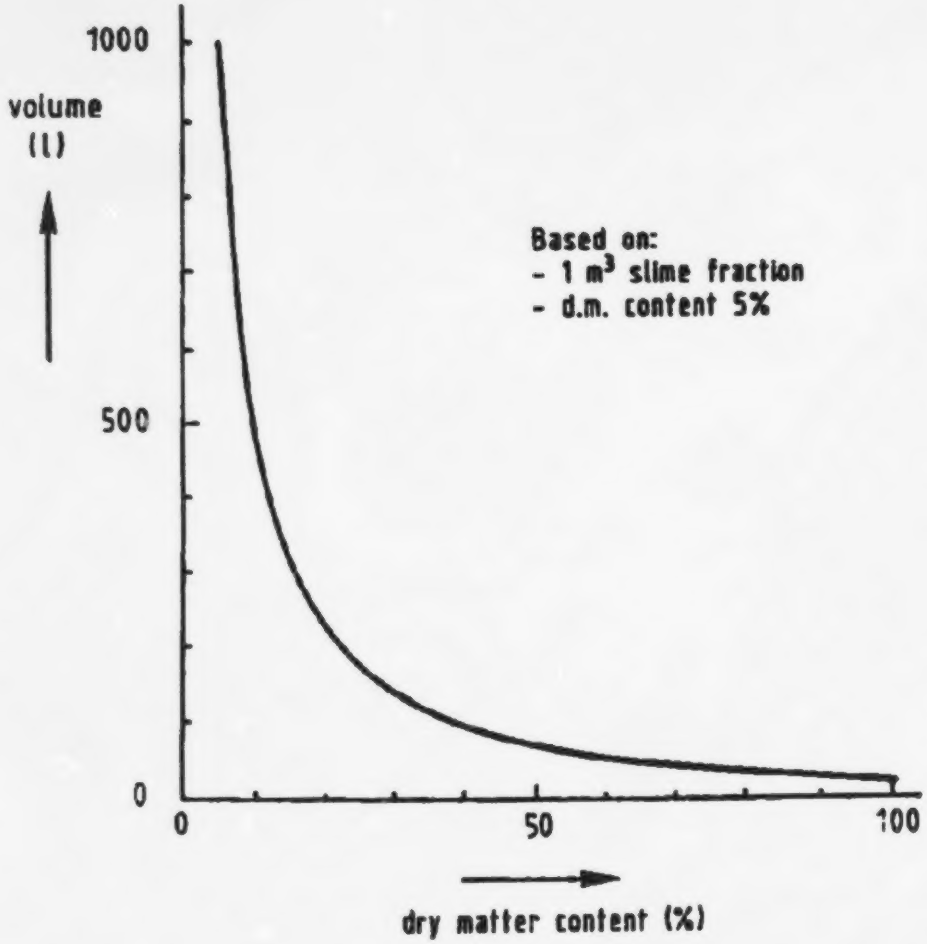


Figure 5.4.3. Volume reduction by dewatering.

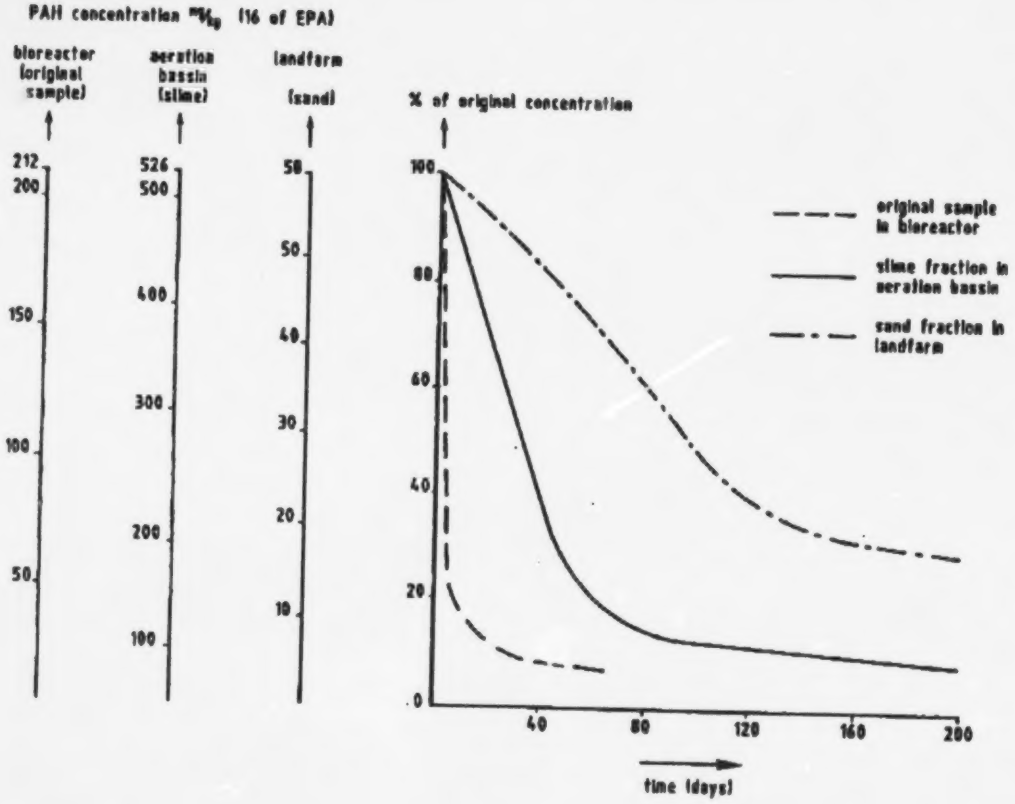


Figure 5.4.4. Intensive versus extensive treatment (Geulhaven Rotterdam).

6 METALS

6.1 Bacterial Leaching of Metals from Various Matrices Found in Sediments, Removing Inorganics from Sediment-Associated Waters Using Bioaccumulation and/or BIOFIX Beads

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INTRODUCTION

The Bureau of Mines Salt Lake City Research Center (SLRC) has been conducting biotechnological research for waste remediation over the past 5 years. Bacteria and immobilized biomass are being used to remove heavy metals and toxic process chemicals from solution; and bacteria are being applied to remediate mining and milling tailings and sediments. Biotechnology is being used by itself and in conjunction with chemical treatments to "polish" solutions to the stringent requirements imposed by environmental legislation. Biotechnology may reduce contaminants to lower concentrations than those achievable using chemical treatment, and may provide on-the-shelf technology for environmental problems untreatable with conventional physical and chemical technology today.

The SLRC has considerable expertise in treating liquid hazardous wastes. Arsenic, cadmium, cyanide, lead, mercury, selenium, and other commonly encountered toxic metals and process chemicals have been removed from a wide variety of wastewaters using both conventional and newly emerging technologies. Conventional techniques utilized include chemical precipitation, ion exchange, and solvent extraction. Newly emerging technologies under investigation at the SLRC include biosorption using viable biological materials such as live bacteria and algae, and immobilized biomass. These latter techniques are currently being addressed by ongoing projects, and have been particularly effective in treating liquid wastes containing dilute concentrations of toxic metals.

Research is being expanded to include bioleaching of inorganic contaminants from sediments and tailings using bacteria. This approach has potential to clean up one of the largest contamination problems in the United States. Tailings are contaminating a large portion of the waterways in the West, especially in Montana. We are currently investigating several biotreatment techniques for the mixed tailings-soils along these arsenic contaminated waterways. Bacteria have been identified which aid in leaching arsenic directly from contaminated soils. Sediments are contaminated with man-made waste throughout the country, and especially in the Great Lakes Region. The nature of these low-level, high-volume wastes makes most processing options extremely expensive. Bacterial leaching in situ or on heap pads may provide an answer to this wide-spread problem.

ONGOING SLRC BIOTECHNICAL RESEARCH PROJECTS

Biohydrometallurgical Decontamination of Mining and Milling Waters

The biohydrometallurgy project develops new biotechnical techniques for decontaminating mining and milling waters containing heavy metal ions and toxic process chemicals. This includes using live bacteria to remove heavy metals, such as arsenic, cadmium, cobalt, chromium, lead, selenium, and zinc from solution, as well as destroying cyanide in solution. Chemical techniques are also investigated, but to a lesser degree, to enhance results. Successful development of the biotechnical techniques will provide alternative remediation technology to those available today for liquid wastes including those that must be treated during heap leach closure.

Immobilized Extractant Technology for Wastewater Treatment

The immobilized extractant technology project is investigating procedures to immobilize biological materials in polysulfone beads. The beads have excellent handling characteristics and have been utilized to extract toxic metal ions such as cadmium, lead, and mercury from wastewaters. Successful application of this technology will provide an innovative method for removing and recovering heavy metals from a wide variety of mining and mineral processing wastewaters.

Hazardous Wastes on Federal Lands

The federal lands project investigates remediation of hazardous wastes specifically under the jurisdiction of the United States government. Sites under investigation include (1) the inactive Midnite Mine on the Bureau of Indian Affair's Spokane Reservation contaminated with uranium and radium, (2) the Olson-Neihart tailings just outside of Heber, UT, generated by a lead-zinc operation, now under the jurisdiction of the Bureau of Reclamation, and (3) recent involvement with the U.S. Forest Service on permitting gold operations on Forest Service lands including closure technology. Development of this technology could help alleviate the wide-spread hazardous waste problems on federal lands which the Federal Government must remediate.

Technical Consultation and Support

This project provides technical consultation and support to the Bureau and other cooperating agencies in assessment of techniques to decontaminate Superfund and metal mining waste sites. This includes reviewing the technical credibility of recommendations relating to Superfund Sites, such as those included in Environmental Impact Statements (EIS), Remedial Investigations (RI), Feasibility Studies (FS), and Records of Decision (ROD). These manuscripts are reviewed at the request of the Bureau's Washington Office.

Cooperative Efforts with Other Agencies and the Private Sector

Cooperative demonstrations of SLRC developed procedures for wastewater and solids remediation are being conducted with various private and public agencies.

- Memorandums of Agreement (MOAs), for cooperative work, are being signed with the mining industry for bacterial cyanide destruction in mine tailings ponds and in spent ores.
- A blanket MOA is in place between the Bureau of Mines and the U.S. Forest Service for remediation of acid mine drainage waters. Part of the SLRC effort is using immobilized biomass to remove heavy metals from waste waters. Another effort has begun for the SLRC to review of EIS, RI, and FS documents for permitting gold mining and milling operations including closure technology.

- A blanket MOA has been in effect with the Bureaus of Mines and Indian Affairs (BIA), and a specific MOA has been in effect between the SLRC and the BIA in Spokane, WA, for the past 3 years for remediation of Midnite Mine including treatment of 500 million gal of impounded water to meet NPDES limits, checking for the reactivity of rock impounded on site, and reviewing draft closure technology.

- An MOA is in place with the Colorado Department of Natural Resources for using immobilized biomass to remove heavy metals from waste waters.

- An MOA has been completed with the Bureau of Reclamation out of Sacramento, CA, for bacterial removal of selenium from agricultural drainage waters.

- An MOA is nearing completion with the Bureau of Reclamation out of Provo, UT, for determining chemical and biological oxidation of Olson-Neihart mine tailings (once proposed to be proposed for the NPL list) during drying and relocating in a new impoundment Heber, UT.

- Over the past 4 years we have reviewed treatment alternatives in various EIS, RI, FS, and RODs at the request of our Washington Office under an MOA between the Bureau of Mines and USEPA.

BIOREMEDIATION OF LIQUID WASTE

Background

The bioaccumulation of metals is the reverse reaction of bioleaching; instead of mobilizing metals from minerals, microorganisms remove soluble metal ions from contaminated water. Bioaccumulation has received considerable attention in the scientific community. Several important conclusions are evident:

- Bioaccumulation is effective, often superior to conventional metal removal systems such as solvent extraction or ion exchange.

- Bioaccumulation processes can be applied to a wide variety of metals. In this report, cadmium, cobalt, nickel, zinc, and uranium will serve as prominent examples, although other metals are known to be susceptible to bioaccumulation and will be mentioned where appropriate.

- A variety of biological mechanisms play a role in these processes. In some cases, the bioaccumulation of a metal involves the active uptake of the metal into the cell; in other situations, passive adsorption of the metal to the cell wall may occur; still another mechanisms deals with the complexing of metals by specific metabolic binding wastes such as H_2S. There are approximately a dozen recognized metal accumulating mechanisms.

- Metal removal processes can be devised to make use of live and dead cells. In many cases, the use of bioaccumulation using non-viable cells is as effective or more effective than using living cells.

- Bioaccumulation has a recognized, established use in the mining industry; Schist Lake, Manitoba, and various other sites, make use of microbial systems to remove metal pollutants (generated from mining activities) from surface waters.

Specific Examples Cited in the Literature

Cadmium

Reports of environmental cadmium contamination of waters has dramatically increased over the last quarter of a century. The Marathon Battery site, located near Buffalo, NY is a notorious example. This heavy metal presents a variety of environmental problems; it is among the more toxic elements, and is readily accumulated by many living organisms, including man. John Poldoski, EPA ERL, studied the possibility of cadmium accumulation by the microorganism *Daphnia magna* in the late 1970s (*46*); however, much of the cadmium bioaccumulation research originated in England. In 1982, researchers at Oxford University reported a novel approach for cadmium removal (*38*). These scientists selectively altered the enzymatic pathway of a bacterial strain belonging to the enteric family. This strain of *Citrobacter* was engineered to produce a cellular phosphatase at levels well above its normal range. This enzyme cleaves organic phosphate, yielding HPO_4^{2-} which then binds soluble cadmium to the cell membrane as insoluble cadmium phosphate (*39,42*). The SLRC performed some exploratory research on Marathon Battery Superfund sediments in 1986 (*4*). Research focused on using such an enzyme system produced by *Pseudomonas aeruginosa* to remove heavy metal ions from contaminated ground water.

The same enzyme-metal binding pathway has been reportedly used by the same bacteria for lead (*1,2,3*), strontium (*40*), and uranium (*41*).

Other cadmium removal mechanisms exist. Two species of yeast accumulate cadmium through a biosorption mechanism: *Aureobadisium pullans* (*43*) and *Saccharomyces cerevisiae* (*44*). This biosorption mechanism is explored below in the section on cobalt and nickel recovery.

Cobalt and Nickel

Cobalt has many important uses in industry and is considered a strategic metal. Cobalt is frequently found in nickel-bearing deposits. To quote Brierley, "Advances in biomining technology may make it possible to recover not only some of the nickel (worth $60 billion at 1982 prices) but also some of the cobalt ... the emphasis is not so much on rapid reaction rates as it is on lower capital investment, greater recovery of metal, and reduced environmental damage" (*13*). The need for increased bioaccumulation research is clear; as Brierley points out, biological accumulation of metals may be relatively cheap and effective.

Investigation of cobalt bioaccumulation is not new. In 1954, Parker and O'Brien studied the bioaccumulation of cobalt by *Saccharomyces cerevisiae*, common brewer's yeast. They found a cobalt resistant strain that would accumulate the metal ion at uptakes of 10 pct of the dry weight of the organism. The cobalt accumulating ability of *S. cerevisiae* was verified in 1977 by Norris and Kelly (*44*) as was mentioned in the previous section on cadmium.

Kuyucak and Volesky (*28*) reported a recovery system that used microalgae to remove cobalt from solution. Their process is complex, but works extremely well. The mechanism, biosorption, works as follows: The cell wall of many microorganisms is porous to allow uptake of organic nutrients and trace minerals. Metal ions enter cells through the same porous channels, where they can bond to a variety of anionic cellular components such as sulfhydryl groups, phosphate groups, amino acids, or polysaccharides. These anionic cellular components act as electrostatic magnets for a number of metal cations including nickel, lead, zinc, chromium, copper, iron (*28*), uranium, thorium (*48*), germanium (*17*) and gold (*29*). Biosorption is a phenomenon that has been linked to many bacteria and yeasts, as well as algae.

The rate of metal recovery using biosorbants compares favorably to ion exchange systems, often working faster, and removing more metal from solution while costing less than the conventional technology. As Brierley also notes, three metal removal systems were in effect in 1982 that made use of such biosorption mechanisms; at Schist Lake in Manitoba, at the New Lead Belt in Missouri, and at the Grants Uranium District in New Mexico the mining industry has used the accumulating ability of various microorganisms to remove a wide variety of soluble, toxic metal cations (*13*).

Kuyucak and Voleski (*28*) also point out that the sorbed metals could be recovered through chemical stripping, allowing the metals to be sold to help offset costs. Their process also illustrates that living and dead algal biomass could be reused, without losing its effective metal binding properties. This supports results from the immobilized biomass bead research being conducted at the SLRC today. This new technology may make Brierley's comments a reality.

Zinc

Zinc is a trace nutrient for most living systems; however, it has been demonstrated that a number of microbial fungi can accumulate the metal. White and Gadd (*50*) explored the uptake and intracellular accumulation of zinc by *S. cerevisiae*. They found that this useful microorganism accumulates the metal in a two-step, energy dependent reaction.

The mechanism behind the accumulation of metals by *S. cerevisiae* has been studied; the phenomenon may be related to the presence of an intracellular binding protein such as metallothionine. These proteins are high in sulfhydryl groups and have significant metal binding properties. Bacteria, yeasts, and molds are all capable of forming metallothionines in response to the presence of heavy metals. The specific zinc binding metallothionine of *S. cerevisiae* has been documented by researchers at the University of Utah (*51*).

A high affinity zinc accumulation system was demonstrated using the yeast *Candida utilis* (*20*). The yeast cells were grown under abnormally low zinc conditions; however, when the cells were exposed to higher levels of the trace element, they hyperaccumulated the metal - almost 10 times the normal level - up to 1 pct of the dry weight biomass.

Researchers at the Bureau of Mines, SLRC, have developed an effective bioaccumulating system that removes zinc (and most other divalent metal cations) from aqueous solutions (*12,30*). Their research has explored the sorptive powers of a number of biomass sources, both living and dead. The procedure which they have developed works well and has been effectively tested on the heavy metal tainted waters from near Leadville, Colorado. This work followed the success of Darnall and others (*18*) who effectively removed zinc, uranium, barium, gold, and other metals from solution using immobilized microalgae.

Uranium

Uranium has been mentioned throughout this report; much of the conventional uses of bioaccumulation have focused on the recovery of this metal. Uranium, found principally as hexavalent uranium (U^{+6} present as UO_2^{2+}), is among the easiest of metals to remove by biosorption (*18,49*). Tsezos evaluated the uranium accumulating ability of a variety of molds and bacteria; he applied the same technology to the removal of thorium and radium.

As mentioned previously, Macaskie and Dean (*40*) used the same system to remove cadmium and uranium from waters with the uranium precipitating as cell-bound uranylphosphate.

A third uranium removal system demonstrates a different way that microorganisms remove metal cations from aqueous systems: by the production of metabolic wastes. Near Ambrosia Lake, New Mexico, uranium mine discharge water is percolated through soil. The water contains elevated molybdenum, selenate, and sulfate levels as well. The removal of the minerals from the water is due to the presence of soil bacteria, most notably *Clostridium* and *Desulfovibrio*. These bacteria metabolize the sulfate and selenate to hydrogen sulfide and elemental selenium, respectively (*27*). The hydrogen sulfide reduces the uranium to insoluble uranium dioxide and binds the molybdenum as insoluble MoS_2. In this case, the treatment is effective in reducing the level of all of the minerals below required levels.

The importance of this project is that conventional treatment technology was ineffective in removing the metals from solution; however, applied microbiology did the trick. It can be postulated, based on this model, that other soluble metal cations can be removed from solution in a similar manner. Cadmium, cobalt, lead, mercury, nickel, tin, zinc, and other metals are subject to the binding power of hydrogen sulfide, precipitating as insoluble metal-sulfides. With a careful design, it would be possible to remove these metals from solution and recover the metals for future refinement.

Examples of SLRC Biotechnical Removal of Organics from Contaminated Waters

Bacterial Cyanide Destruction and Selenium Removal From Precious Metals Solutions and Tailings

Ponded cyanide solutions in the environment pose a serious threat to migratory birds and are responsible for several bird kills each year. An additional threat is posed by selenium, which is also present in some of these solutions. While selenium is a necessary nutrient in trace amounts, high concentrations cause death and deformities in wild fowl. The Bureau is conducting research to reduce costs of cyanide destruction and selenium removal. Presentations at both the TMS and SME Annual 1990 Meetings describing the Bureau's research on cyanide and selenium removal from tailing waters generated considerable interest (*5,8,34,35,36,37*). More problems exist with selenium than was originally thought. In a number of precious metal operations, both cyanide and selenium are potential problems and operators need information on how to deal with them. As an alternative to expensive chemical destruction of the cyanide, the Bureau has cultured and isolated cyanide-destroying bacteria from toxic, pH 10.5 precious metals tailings pond water containing 280 ppm CN. The cyanide-destroying bacteria are *Pseudomonas pseudoalcaligenes* and *Pseudomonas diminuta*. The bacteria have been oxidizing 85 to 95 pct of the cyanide from two cyanide solutions obtained from different industrial operations for over a year in a continuous system. Results from treating one water are shown in Figure 6.1.1. In addition to degrading the cyanide, the bacteria also remove other contaminants. Most of the iron, lead, nickel, and zinc are removed; however, copper, selenium, and silver are not. Selenium is the major remaining contaminant. Selenium can be chemically precipitated from solution using copious amounts of ferrous sulfate, upwards of 600 times the stoichiometric amount, to approach the drinking water standard of 10 ppb. Once again, bacterial treatment was investigated, but no selenium-reducing bacteria were found in this toxic precious metals solution. Luckily, earlier SLRC research involved the selenium-contaminated waters of the Kesterson Reservoir, located in the San Joaquin Valley of California (*9,32,33*). Of all the selenium-reducing bacteria isolated, *Pseudomonas alcaligenes* reduce selenium fastest under anoxic conditions. After the cyanide is destroyed, these bacteria reduced the selenate to selenite and then to elemental selenium which precipitates from solution as a red amorphous mass. These promising results may provide effective technology for application during heap leach closure for precious metals operations. This technique might also have application to remediation of plating waste sediments and associated waters.

Arsenic Removal Using Anaerobic Bacteria

SLRC researchers isolated anaerobic bacteria that reduce arsenic and precipitate it from solution. Continuous and batch tests are ongoing to optimize parameters and devise an operational treatment system. Arsenic removal of 23 pct has been achieved in the continuous system, and upwards of 70 pct of the arsenic has been removed in batch tests (*6*).

Cadmium Removal Using Aerobic Bacteria

Bacteria that reduce cadmium from solution in the presence of nickel and cobalt were isolated from sediments in the Marathon Battery Superfund Site.

Metal Contaminant Removal Using BIOFIX Beads

The SLRC has developed a material which utilizes immobilized biomass for removing metal contaminants from a wide variety of mining and industrial wastewaters (*22*). The original objectives of this work were to produce a material compatible with conventional equipment and procedures, produce a reusable and easily regenerated material, and recover the sorbed metal ions. These objectives have been met and have resulted in polymeric beads designated as BIOFIX beads (*11,21,30*). These beads are being awarded an R&D 100 award in 1990 as one of the 100 most valuable domestic inventions by Research and Development Magazine.

The beads, which are spherical in shape and somewhat similar in appearance to ion exchange resins, can be produced in a variety of sizes depending on the targeted application. BIO-FIX beads are produced from readily available raw materials including high-density polysulfone pellets, an organic solvent, and dried, thermally-killed biomass obtained from microorganisms and aquatic flora.

The fabrication procedure consists of dissolving polysulfone in the organic solvent, blending dried, minus 100-mesh biomass into the polymer solution, and spraying fine droplets of the mixture into water. Spherical beads are immediately formed, and are ready for use after curing in water for 12 to 16 hours. The cured beads are porous, resistent to attrition, and stable in strong acid and base solutions.

Types of biomass immobilized in the beads include algae, common duckweed, peat moss, and other materials. Sphagnum peat moss has been the most effective material utilized thus far, and has the added advantage of being abundant and inexpensive.

An attractive feature of the beads is that their effectiveness in sorbing the metal contaminants most frequently encountered in mining and industrial wastewaters: cadmium, mercury, lead, etc. Although sorption of these metal ions is a characteristic of the individual biomass used, some materials, particularly peat moss and certain algae, will remove most of these metals from wastewaters. Evidence of the effectiveness of the beads for removing metal ions from dilute wastewaters is shown in Figure 6.1.2. Cadmium, copper, manganese, and lead were removed from various waters, and in each case the resulting effluent met National Drinking Water Standards. These tests were conducted in fixed-bed columns and stirred tanks, and biomass types utilized included peat moss and 2 species of algae. Contact times were 5 to 10 minutes in each test.

An important feature of BIO-FIX beads is that sorbed metals are readily eluted of sorbed metals using dilute mineral acids. Since only a small volume of acid is required for elution and regeneration, significant concentration of the metal values is possible. As an example, acid mine drainage water containing 10.5 parts per million zinc and 4.3 parts per million manganese was processed in a 3-column fixed-bed circuit. Over a period of several loading-elution cycles, the effluent consistently met all discharge standards, and elution with 20 g/L sulfuric acid produced an eluate containing about 100 times as much zinc and manganese as the original wastewater. Subsequent tests indicated that this eluate could be further concentrated using conventional hydrometallurgical techniques for eventual recovery of the metal values.

Although most of the work with BIO-FIX beads has involved conventional processing equipment, recent laboratory and field tests have indicated the potential for use of the beads in passive systems having low maintenance and labor requirements. One promising technique consists of enclosing the beads in porous bags fabricated from polypropylene, placing the bags in a natural trench or constructed trough, and allowing wastewater to flow through the bags by gravity. Periodically, the beads most fully loaded with metal ions would be collected and replaced with fresh beads. The loaded beads would then be regenerated on site or transported to a central location and regenerated. This type of system may be especially useful for treating small seeps where conventional technologies are often difficult and expensive to apply. Tests have indicated that beads enclosed in porous bags exhibit the same loading and elution characteristics as beads utilized in other equipment.

The development of BIO-FIX beads has resulted in a material well-suited for removing metal contaminants from mining and mineral processing wastewaters. The beads are fabricated from easily obtained raw materials, accommodate a wide variety of biomass, have excellent handling characteristics in conventional equipment, and demonstrate long-term chemical and physical stability. In addition, the beads readily sorb metal contaminants from dilute solutions, selectively sorb toxic and heavy metals over calcium and magnesium, exhibit good sorption and elution kinetics, and are readily eluted and regenerated.

BIOREMEDIATION OF SOLID WASTE

Background

One of the oldest, most studied methods of removing metals from rocks and soils is leaching. There are two principle types of mineral leaching: chemical and biological, however, the boundary between the two is often obscure. Microbes (bacteria and molds) are able to mobilize metals from rocks and sediments through a variety of ways, but the best studied examples link metal leaching to the production of metabolic waste acids: nitric, sulfuric, or a wide variety of organic.

The familiar example of bioleaching is found in the copper industry. The soil bacteria *Thiobacillus ferrooxidans* oxidizes sulfide minerals to sulfuric acid, in the process metallic ions are liberated. These metals can subsequently be concentrated from heaps of rock ore and collected for refinement. It has been estimated that 20 pct of all copper mined in the U.S. is recovered from bioleaching operations. Thiobacillus mediated leaching is also important in the recovery of domestic uranium and other non-ferrous metals. A more detailed study of sulfide oxidizing bacteria will be examined later in the report.

This example has served as a model to illustrate the potential for other bioleaching operations. Many minerals are subject to biological mobilization, however, the possibilities for bioleaching are barely being recognized. Most of the actual applications of bioleaching have taken place to enhance mineral recovery from low yield ores. These ores are frequently unamenable to any other treatment.

Information on the use of bioleaching to remove metal pollutants from soils and sediments is scarce, however, the possibility for this type of application is bright. The same treatment systems that are now used to recover uranium for the mining industry would work equally well to remove metallic wastes. The reason is simple: Biotechnology is flexible. The appetite of leaching bacteria is fairly non-specific. The same bacteria that mobilize copper from sulfides also mobilize zinc, lead, and other metals in the process. Microorganisms have diverse appetites; they are capable of deriving energy from the degradation of carbonates, phosphates, sulfides, oxides and other minerals - liberating metals in the process.

As seen in the mining industry, practical applications of bioleaching are relatively inexpensive and fairly easy to maintain. In situ leaching of metal pollutants may be possible with the simple addition of a microbial nutrient source. In other cases, where metal toxicity would be expected to disrupt a biological setup, direct contact between the bacteria or mold and the metal would not be required. A simple, two-step operation may be possible: First, the production of microbial-generated metabolic acids; two, application of the leach solution to remove the metals from tainted sediments.

Specific Examples in the Literature

Sulfides

This example is detailed to show the wide range of metals that can be mobilized through bioleaching. As stated in the introduction, a large portion of the domestic copper market is filled by the recovery of bioleached copper. The mechanics of this leaching are well studied (*14*). Many bacterial groups are capable of degrading sulfide sources including *Thiobacillus*, *Sulfolobus*, *Thermothrix*, and *Leptospirillium*. These bacteria derive energy from the oxidation of sulfide minerals such as covellite (CuS), chalcopyrite ($CuFeS_2$), and pyrite (FeS_2). Some members of these groups find uses for the metallic component of the mineral as well: *Thiobacillus* is known to get electrons from the oxidation of ferrous iron, *Sulfolobus* uses molybdenum as a metabolic electron sink, reducing Mo^{6+} to a lower valence (*13*).

The usual metabolic waste product from sulfide degradation is sulfuric acid. A wide variety of metals, other than copper, can be liberated by this process. Bioleaching has been applied in Canada since 1971 to recover uranium from ores that contain minute traces of the metal (*23*). Several projects have been conducted in the U.S. and Canada since that time (*31*). Precious metals, gold, silver, and platinum-group metals are being subjected to bioleaching prior to cyanidation. The metals are often found as discreet metal-sulfides; this pre-treatment makes the metals more responsive to recovery than can be achieved through only chemical means (*16*).

Sphalerite (ZnS), galena (PbS), cinnabar (HgS), and pentlandite $[(Fe,Ni)_9S_8]$ yield soluble zinc, lead, mercury, and nickel when subjected to bioleaching. Cobalt is frequently recovered in trace amounts from nickel ores (*13*).

These examples show that bioleaching is being applied to enhance metal recovery from mining operations. It would not, however, be difficult to modify these systems to recover metal pollutants from sediments. Figure 6.1.3 shows a conceptual configuration for bioleaching sediments based on industrial leaching of copper ores.

Carbonates and Phosphates

The ability of microbes to attack carbonate and phosphate minerals is an important part of the natural cycling of these elements; the specific details of these cycles can be found in texts on geochemistry or microbial ecology. These microorganisms are able to solubilize insoluble phosphate and carbonate deposits through the production of acidic metabolic end-products that lower pH and attack the metal-anion bonds.

Calcareous minerals (limestones) can contain elevated concentrations of certain metals. The shellfish and coral that originally made up these sediments are capable of concentrating metals many thousand times over surrounding levels. When these marine animals died, the metals they had accumulated were trapped in a calcium carbonate matrix. Acidic metabolites, produced by bacteria and molds dissolve the carbonate material, eventually yielding carbon dioxide and a residual cationic metal (Ca^{2+}). The trace metals which were trapped in the sediments are liberated in the process. As an example, selenium is found in abundance within shale deposits of the San Joaquin Valley of California. This selenium is being mobilized from the exposed shale as the carbonaceous minerals are subjected to biological (and natural) weathering. This mobilization has been linked to problems associated with high selenium levels in that area.

The cycle for phosphorus is similar to the cycle for carbonate. Calcium phosphate is vulnerable to a variety of organic acids produced by bacteria, formic, oxalic, and citric acids being notable. Ferric and other metal phosphates are subject to the metal-liberating power of hydrogen sulfide, a common bacterial metabolite. Thus, rocks or sediments that contain metal phosphates would be susceptible to bioleaching.

Silicates

Little information is available on the leaching of silicate ores, although a good example has been provided from the Soviet Union (*26*). Spodumene, $LiAlSi_20_6$, has been subjected to the solubilizing power of biologically generated organic acids; the process liberating the lithium and aluminum. Other authors have investigated the application of biotechnology to remove silicate from low grade aluminum ores (*24*). The mechanism(s) behind leaching of silicates are not known, however, this illustrates the usefulness and wide range of applied microbiology.

Oxides

Research on the reduction of metal-oxides shows the applicability of microbial systems. Manganese is an important non-ferrous metal that is being recovered through the use of microbial geochemical agents. Many bacteria are capable of reducing MnO_2 if they are provided with an oxidizable nutrient source. There are several ways that MnO_2 can be reduced.

First, the metal-oxide can serve as a terminal electron acceptor for respiratory enzymes, replacing oxygen. The model for this example is:

$$RH_2 + MnO_2 \longrightarrow Mn(OH)_2 + R$$

Enzyme preparations that accomplish this reduction were isolated by Bautista and Alexander in 1972 (*10*). A large number of prominent bacterial groups have demonstrated this ability, including species of *Bacillus*, *Clostridium*, *Micrococcus*, and *Pseudomonas*. Several molds have also shown this enzymatic capability.

Second, as with the carbonates, phosphates and silicates, biological metabolic waste acids are effective in leaching manganese from oxide materials. This reaction is:

$$\underset{(MnO_2)}{Mn^{4+}} \xrightarrow{H^+} Mn^{2+}$$

The hydrogen ion being provided by bacteria and other microbes. Paponetti (*45*) reported on the recovery of manganese using citric acid produced by the mold *Aspergillus niger*. Gupta and Ehrlich (*25*) used a mixed microbial culture to remove manganese from silver ores, prior to cyanidation, because the manganese interferes with the silver recovery process. [Ehrlich used a similar experiment to demonstrate the possibility of bioleaching nickel and cobalt from sea nodules (*19*)].

Researchers at the Bureau of Mines Reno Research Center have studied the reduction of manganese using a species of *Thiobacillus*. Information on this research will be presented in this session. This bacteria is able to solubilize manganese from oxides by a pathway that is similar to it's other metal liberating systems. *Thiobacillus thiooxidans* oxidize sulfur compounds in sediments and ores, producing sulfuric acid. The acid effects the reduction of manganese from the insoluble 4+ state to the soluble 2+ state. There is a curious highlight to this biological mediated leaching - the bacteria were able to solubilize more manganese than could be achieved by use of non-bacterial generated sulfuric acid. It was concluded that the difference may be that the bacteria are able to liberate more manganese because they are additionally using the Mn^{4+} ion as a terminal electron acceptor.

Exploratory SLRC Biotechnical Research on Great Lakes Sediments

Preliminary research into the possibility of removing heavy metal contaminants from Great Lakes sediments through biotechnology is encouraging. Experiments being conducted at the SLRC are based on the following premise: that many bacteria produce organic and inorganic acids as a byproduct of metabolism. These acids can be successfully used to leach metals from minerals and sediment compounds.

This experiment has a precedent. In a previous test, manganese, cobalt, cadmium, and lead were successfully leached from simulated Great Lakes sediments using the system described above. Simulated Great Lakes sediments were used due to the lack of actual sediments and were created by crushing sea nodules. The sea nodules are rich in insoluble manganese and cobalt compounds; to this artificial sediment, insoluble cadmium and lead salts were added. The organic acids were produced by a species of *Klebsiella*, a member of the enteric family.

The Grand Calumet/Indiana Harbor (GC/IH) sediments were used as a model for these tests. From the GC/IH site, a wide variety of bacteria were cultivated that could possibly be used to produce acids which would leach the metals from the sediments. The GC/IH site is rich in organic waste (sewage, oils, and aromatic compounds) and attempts are underway to determine if bioleaching can be accomplished using these on-site bacterial feed compounds. This would alleviate the cost of adding a "bulk" carbon compound nutrient, such as sugar. The Saginaw River (SR) and Buffalo River (BR) sediments have received little attention as yet, however, it is felt that any remediation system that is devised for the GC/IH site would apply equally well to these other two sites.

Identification of Bacteria From the Grand Calumet/Indiana Harbor, Saginaw River, and Buffalo River Sediments

Research is being conducted at the SLRC under an MOA with EPA on beneficiation of GC/IH, SR, and BR sediments for decontamination. Small samples of these sediments were obtained by the Biotechnology Group for exploratory studies. A population study was begun on the sediments from the various sites to determine the types of bacteria present. (This study made no attempt to account for molds or viruses in the sediment slurry.) There were two reasons for checking the bacterial types found at the sites. First, we needed to establish if the bacteria found in the sediments were capable of producing acid metabolites that could leach the heavy metals. Second, we needed to determine if any potential biological health hazard exists in working with the sediments. This analysis was performed when it was discovered that a large portion of the organic sediment at the GC/IH site is sewage.

The population study of the Grand Calumet sediments revealed an extremely diverse collection of microorganisms. Aerobic, facultative, and anaerobic bacteria were all identified; many of the bacteria encountered are typical water and sediment types including several species of *Pseudomonas* (*P. aeruginosa*, *P. fluorescens*, and *P. putida*), a *Bacillus* (*B. subtilis*), *Acinetobacter anitrus*, and a sulfate reducing bacteria (probably *Desulfovibrio*). *Clostridium sporogenes*, and anaerobic sediment bacteria, was also identified. Tests revealed approximately $5x10^{10}$ bacteria/mL in the sediments sample we received (the sediment sample was 40-pct solid).

Total coliform and fecal coliform tests were run to determine the level of bacteria introduced to the GC/IH site through sewage run-off; the numbers were **$3x10^6$** coliform/mL and **$1.8x10^4$** fecal coliform/mL respectively. These last two numbers show the seriousness of the biological contamination of the GC/IH site; the National Drinking Water Standard allows for only 1 coliform per 100 mL of water. The coliforms that were identified included *E. coli*, *Enterobacter cloacae*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Salmonella enteritis* and *Streptococcus faecalis*. *Staphylococcus aureus* as well as a *Haemophilus sp.* were also identified. These last four bacteria are potential pathogens. Other bacteria suspected as present, but not confirmed include *Methylococcus*, *Aeromonas*, and *Selenomonas*. These tests were run at the SLRC and verified, independently, by the Utah State Health Laboratory. Identification on GH/IH bacteria is continuing.

The Saginaw River (SR) and Buffalo River (BR) sites were similar to the GC/IH site in many respects. The total cell counts were similar and all three sites contained many of the same water and sediment bacteria including *Bacillus* and *Pseudomonas* species. One important difference, however, the coliform tests for the SR and BR sediments showed that these waters are below the NDWS guidelines for coliform bacteria.

From the results of the identification work, two conclusions were reached. First, that a low level health hazard existed which could be remedied through safe-handling techniques (minimization of contact and washing). Second, that acid producing bacteria, such as *Bacillus*, were endemic to the sediments.

Bacteria in De-oiled Sediments

The first step in beneficiation research being conducted at the SLRC is de-oiling the sediments. Bacteria were also cultured from GC/IH sediments which had been de-oiled with (1) a double methanol wash, followed by (2) repulping in equal volumes of methanol and refiltering, followed by (3) drying at 105° C, followed by (4) soxhlet extraction with 1,1,1 trichloroethane, followed by (5) drying at 105° C. The process of de-oiling the GC/IH sediments appears to have eliminated the vast majority of bacteria that were found in the sediments. Two bacteria, *Bacillus subtilis* (a spore forming bacteria) and a strain of *Pseudomonas*, probably *P. aeruginosa*, were isolated from a sample of de-oiled sediments however the bacteria were not very numerous.

Inorganic Leaching Capability of Indigenous Bacteria

The objective of the exploratory studies currently being conducted is to determine if the bacteria will use the organics present in the sediments as a nutrient, or if they need a supplemental nutrient. A batch experiment was set up using GC/IH sediments; 18 samples were placed in flasks with an equivalent amount of water (to make sampling easier). Two sterile controls were produced by autoclaving those flasks to kill the microorganisms present. Two other flasks were sterilized and were then inoculated with *Enterobacter* and *Bacillus* species to determine how well these single species would leach metals. *Enterobacter* was chosen because it is closely related to the bacteria from the synthetic soil experiment which was described above. *Bacillus* was chosen because it is a good acid producer and it is tolerant of heavy metals. The remaining flasks were not autoclaved. Six of the flasks contained only the diluted GC/IH sediments to determine if the organics present in the sediments could serve as a source of bacterially generated acids. To the remaining nine flasks, sugar (dextrose) was added in different concentrations. Results are pending chemical analysis.

CONCLUSION

We think that bioremediation of inorganics in sediments shows potential. Successful development of the biotechnical techniques may provide on-the-shelf technology for environmental problems untreatable with conventional technology today.

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REFERENCES

1. Aickin, R.M. and A.C.R. Dean (1977). Lead Accumulation by Microorganisms. *Microbios Letters*, **5:** 129-33.

2. Aickin, R.M. and A.C.R. Dean (1979a). Lead Accumulation by *Pseudomonas fluorescens* and by a *Citrobacter sp.* *Microbios Letters*, **9:** 55-66.

3. Aickin, R.M. and A.C.R. Dean (1979b). Electron Microscope Studies of the Uptake of Lead by a *Citrobacter sp.* *Microbios Letters*, **9:** 7-15.

4. Altringer, P.B. Biohydrometallurgical Methods for Metals Removal. Presented to USEPA, NYEPA, and EBASCO, New York, NY, Nov. 13, 1986.

5. Altringer, P.B. Biological Cyanide Destruction and Selenium Removal From Precious Metals Solutions. To be presented at the 1990 Intermountain AIME/SME Minerals Conference, Vail, CO, Aug. 9-10, 1990a.

6. Altringer, P.B. and B.E. Dinsdale. Biological Arsenic Removal From Mining and Milling Waters by Anaerobic Sulfate Reducing Bacteria. To be presented and published in the 1991 SME Annual Meeting and Exhibit, Environmental Management Symposium, Water Quality Concerns in the Mining Industry Session, Denver, CO, Feb. 25-28, 1991.

7. Altringer, P.B., R.H. Lien, and K.R. Gardner. Determining Mechanisms of Anoxic Bacterial Selenium Removal. Interagency Agreement No. 9-AA-20-08500 between U.S. Department of Interior, Bureau of Reclamation and Bureau of Mines, April, 1990b, p. 19.

8. Altringer, P.B., R.H. Lien, and K.R. Gardner. Biological and Chemical Selenium Removal From Precious Metals Solutions. To be presented and published at the 1990 SME GOLDTech 4 "North American Practices," Technical Session on "Advances in Gold and Silver Processing", Reno, NV, Sept. 10-12, 1990b.

9. Altringer, P.B., D.M. Larsen, and K.R. Gardner (1989). Bench-Scale Process Development of Selenium Removal From Wastewater Using Facultative Bacteria. **In:** *Biohydrometallurgy*, J. Salley, R. G. L. McCready, and P. L. Wichlacz (eds.), CANMET SP89-10, pp. 643-658.

10. Bautista, E.M. and M. Alexander (1972). Reduction of Inorganic Compounds by Soil Microorganisms. *Proc. Soil Sci. Soc. Amer.*, **36:** 918-20.

11. Bennett, P. G., and T. H. Jeffers. Passive Biological Treatment of Acid Mine Waters. To be presented at the TMS-AIME Annual Meeting, New Orleans, LA, Feb. 17-21, 1991.

12. Bennett, P.G., and T.H. Jeffers (1990). Removal of Metal Contaminants From a Waste Stream Using BIO-FIX Beads Containing Sphagnum Moss. **In:** *Proceedings, Western Regional Symposium on Mining and Mineral Processing Wastes*, F. M. Doyle (ed.), Chapter 35, pp. 279-286.

13. Brierley, C. (1982). Microbiological Mining. *Sci. Am.*, **247:** 44-54.

14. Brierley, C.L. (1978). Bacterial Leaching. *CRC Critical Reviews in Microbiology*, **6:** 207-62.

15. Brierley, C.L. (1982). Microbiological Mining. *Sci. Am.*, **247:** 44-54.

16. Bruynesteyn, A. (1988). Biotechnology for Gold Ores: The State of the Art. **In:** *Proceedings International Gold Conference 88*, Perth, Western Australia, Oct. 28 - Nov. 1, 1988.

17. Chmielowski, J. and B. Klapcinska (1986). Bioaccumulation of Germanium by *Pseudomonas putida* in the Presence of Two Selected Substrates. *Appl. Environ. Microbiol.*, **51:** 1099-1103.

18. Darnall, D.W., B. Greene, M. Hosea, R.A. McPherson, M. Henzl, and M.D. Alexander (1986). Recovery of Heavy Metals by Immobilized Algae. **In:** *Trace Metal Removal From Aqueous Solution: Proceedings Symposium Royal Society Chemistry, Annual Chemical Congress*, R. Thompson (ed.).

19. Ehrlich, H.L., S.H. Yang, and J.D. Mainwaring, Jr. (1973). *Z. Allg. Mikrobiol.*, **13:** 39-48.

20. Faila, M.L. and B.D. Weinberg (1977). Cyclic Accumulation of Zinc by *Candida utilis* During Growth in Batch Culture. *J. Gen. Microbio.*, **99:** 85-97.

21. Ferguson, C.R. and T.H. Jeffers. Biosorption of Metal Contaminants From Acidic Mine Waters. To be presented at the SME Annual Meeting, Denver, CO, Feb. 25-28, 1991.

22. Ferguson, C.R., M.R. Peterson, and T.H. Jeffers (1989). Removal of Metal Contaminants From Waste Waters Using Biomass Immobilized in Polysulfone Beads. **In:** *Biotechnology in Minerals and Metal Processing*, B.J. Scheiner, F.M. Doyle, and S.K. Kawatra (eds.), pp. 193-199.

23. Gow, W.A., H.H. McCreedy, G.M. Ritcey, V.M. Mcnamara, V.F. Harrison, and G. H. Lucas (1971). **In:** *Recovery of Uranium*, International Atomic Commission, Vienna, pp. 195-206.

24. Groudev, S.N. and V. Groudeva (1988). Microbial Removal of Silicon from Mineral Raw Materials. **In:** *Biohydrometallurgy Proceedings International Symposium Warwick*, Norris, P. R., and D. P. Kelly (eds.). Univ. of Warwick.

25. Gupta, A. and H.L. Ehrlich (1988). *J. Biotechnol.*, **39:** 137-42.

26. Karavaiko, G.I., and S.N. Groudev (eds.), (1985). *Biotechnology of Metals*, Moscow.

27. Kauffman, J.W., W.C. Laughlin, and R.A. Baldwin (1986). Microbiological Treatment of Uranium Mine Waters. *Environ. Sci. Technol.*, **20:** 243-48.

28. Kuyucak, N. and B. Volesky. Recovery of Cobalt by a New Biosorbent. **In:** *Proceedings of the Third Annual General Meeting of Biomet.*, R. McCready (ed.) Toronto, Canada, pp. 111-27.

29. Kuyucak, N. and B. Volesky (1986). Recovery of Gold by Biosorption. *Proceedings of the Third Annual General Meeting of Biomet.*, R. McCready (ed.), Toronto, Canada, pp. 171-72.

30. Jeffers, T.H., C.R. Ferguson, and D.C. Seidel (1990). Biosorption of Metal Contaminants using Immobilized Biomass. **In:** *Biohydrometallurgy 1989 Proceedings*, J. Salley, R.G.L. McCready, and P.L. Wichlacz (eds), CANMET, pp. 317-327.

31. Lakshmanan, V.I. (1986). Industrial Views and Applications: Advantages and Limitations of Biotechnology. **In:** *Workshop on Biotechnology for the Mining Industries. Biotech. Bioeng. Symp. 16*, Ehrlich, H.L. and D.S. Holmes (eds.).

32. Larsen, D.M., K.R. Gardner, and P.B. Altringer (1989). Biologically Assisted Control of Selenium in Process Waste Waters. **In:** *Biotechnology in Minerals and Metals Processing*, B.J. Scheiner and F.M. Doyle (eds.), Ch. 22, pp. 177-185.

33. Larsen, D.M., K.R. Gardner, and P.B. Altringer (1987). A Biohydrometallurgical Approach to Selenium Removal. **In:** *American Water Resources Association*, R.F. Dvorsky (ed.), Technical Publication TPS-87-4, Bethesda, MD, pp. 419-426.

34. Lien, R.H., and P.B. Altringer. Biological and Chemical Cyanide Destruction in Heap Leachates and Tailings. To be presented and published in the 1991 SME Annual Meeting and Exhibit, Environmental Management Symposium, Water Quality Concerns in the Mining Industry Session, Denver, CO, Feb. 25-28, 1991.

35. Lien, R.H., B.E. Dinsdale, and P.B. Altringer. Biological and Chemical Cyanide Destruction From Precious Metals Solutions. To be presented and published in the 1990 SME GOLDTech 4 "North American Practices," Symposium on "Advances in Gold and Silver processing", Reno, NV, Sept. 10-12, 1990c.

36. Lien, R.H., B.E. Dinsdale, K.R. Gardner, and P.B. Altringer (1990a). Chemical and Biological Cyanide Destruction and Selenium Removal From Precious Metals Tailings Pond Water. **In:** *EPD 90*, D. R. Gaskell (ed.), AIME-TMS, pp. 323-339.

37. Lien, R.H., B.E. Dinsdale, K.R. Gardner, and P.B. Altringer. Chemical and Biological Cyanide Destruction and Selenium Removal From Precious Metals Tailings Pond Water. Presented at the AIME-SME Annual Meeting, Salt Lake City, UT, Feb. 26 - Mar. 1, 1990b, to be published in Proceedings.

38. Macaskie, L.E. and A.C.R. Dean (1982). Cadmium Accumulation by Microorganisms. *Env. Tech. Letters*, **3:** 49-56.

39. Macaskie, L.E. and A.C.R. Dean (1984). Cadmium Accumulation by a *Citrobacter sp. J. Gen. Microbiol.*, **130:** 53-62.

40. Macaskie, L.E. and A.C.R. Dean. (1985a). Uranium Accumulation by Immobilized Cells of a *Citrobacter sp. Biotech. Letters*, **7:** 457-62.

41. Macaskie, L.E. and A.C.R. Dean (1985b). Strontium Accumulation by Immobilized Cells of a *Citrobacter sp. Biotech. Letters*, **7:** 627-30.

42. Macaskie, L. E., A. C. R. Dean, A. K. Cheetham, R. J. B. Jakeman, and A. J. Skarnulis (1987). Cadmium Accumulation by a *Citrobacter sp*: The Chemical Nature of the Accumulated Metal Precipitate and Its Location on the Bacterial Cells. *J. Gen. Microbiol.*, **133:** 539-44.

43. Mowell, J.L., and G. M. Gadd (1984). Cadmium Uptake by *Aureobasdisium pullans*. *J. Gen. Microbiol.*, **130:** 279-84.

44. Norris, P.R., and D.P. Kelly (1978). Accumulation of Cadmium and Cobalt by *Saccharomyces cerevisiae*. *J. Gen. Microbiol.*, **99:** 317-24.

45. Paponetti, B.L., C. Abbruzzese, A. Marbini, and M.Y. Duarte (1989). Manganese Recovery from MnO_2 Ores by *Aspergillus niger*: Role of Metabolic Intermediate. *Biotechnology in Minerals & Metals Processing*, B.J. Scheiner and F.M. Doyle (eds.), Las Vegas SME Proceedings, pp. 33-37.

46. Poldoski, J.E. (1979). Cadmium Bioaccumulation Assays. Their relationship to various ionic equilibria in Lake Superior water. *Environ. Sci. and Tech.*, **13:** 701-706.

47. Trujillo, E.M., T.H. Jeffers, C.R. Ferguson, and H.Q. Stevenson. Biosorption of Metal Ions on Immobilized Biomass Beads. To be presented at the AIChE 1990 Summer National Meeting, San Diego, CA, August 19-22, 1990.

48. Tsezos, M. and B. Volesky (1981). Biosorption of Uranium and Thorium. *Biotechnol. Bioeng.*, **23:** 583-604.

49. Tsezos, M. (1984). The Selective Extraction of Metals From Solution by Microorganisms - A Brief Overview. *Can. Met. Quart.*, **24:** 141-44.

50. White, C. and G.M. Gadd (1987). The Uptake and Cellular Distribution of Zinc in *Saccharomyces cerevisiae*. *J. Gen. Microbiol.*, **133:** 727-37.

51. Winge, D.R., K.B. Nielson, W.R. Gray, and D.H. Hamer (1985). Yeast Metallothiones. *J. Biol. Chem.*, **260:** 14454-70.

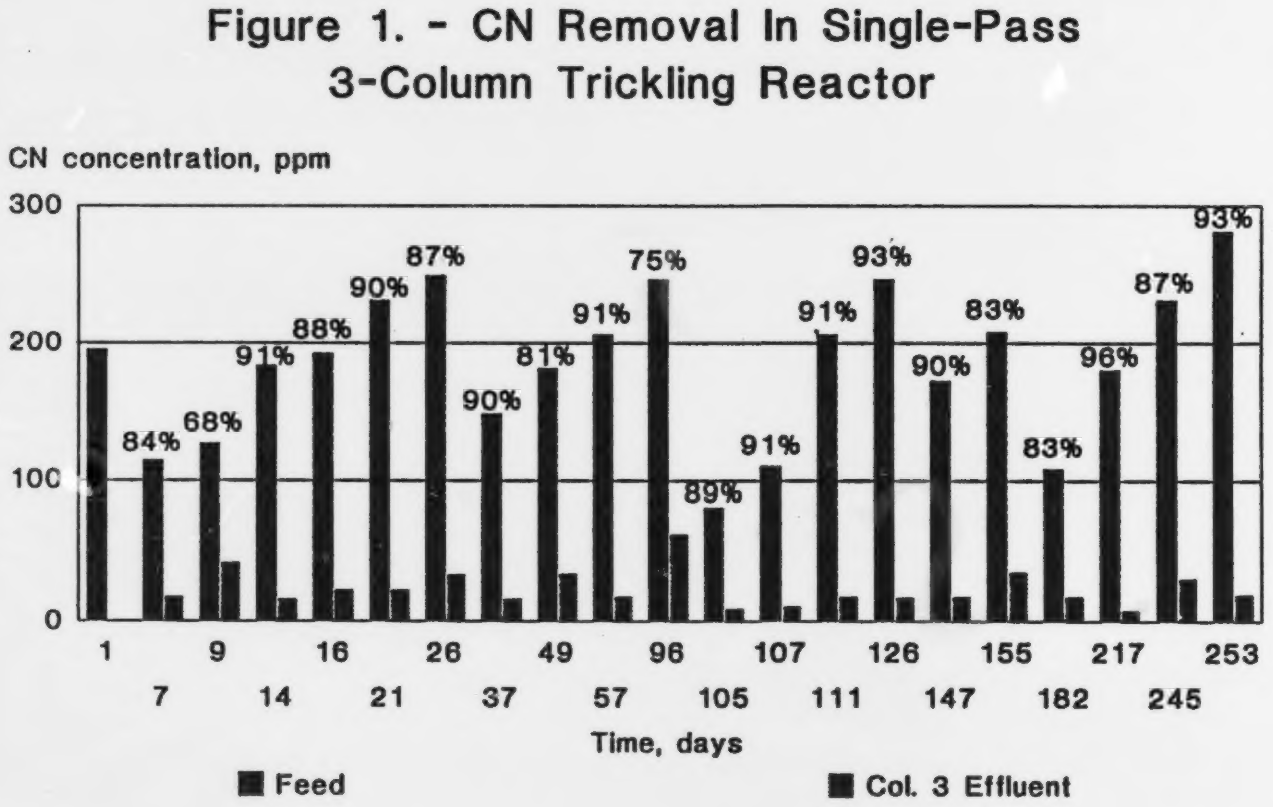

Figure 6.1.1. CN removal in single-pass 3-column trickling reactor.

Figure 2. - Metal Sorption Using BIO-FIX Beads

	Metal Concentrations, mg/L			
	Waste Water	Treated Effluent	National Drinking Water Standard	Metal Removal, pct
Cadmium	0.060	0.001	0.01	98
Copper	2.0	0.023	1.0	99
Manganese	4.7	0.018	0.05	99
Lead	0.059	0.002	0.05	97

Figure 6.1.2. Metal sorption using BIO-FIX beads.

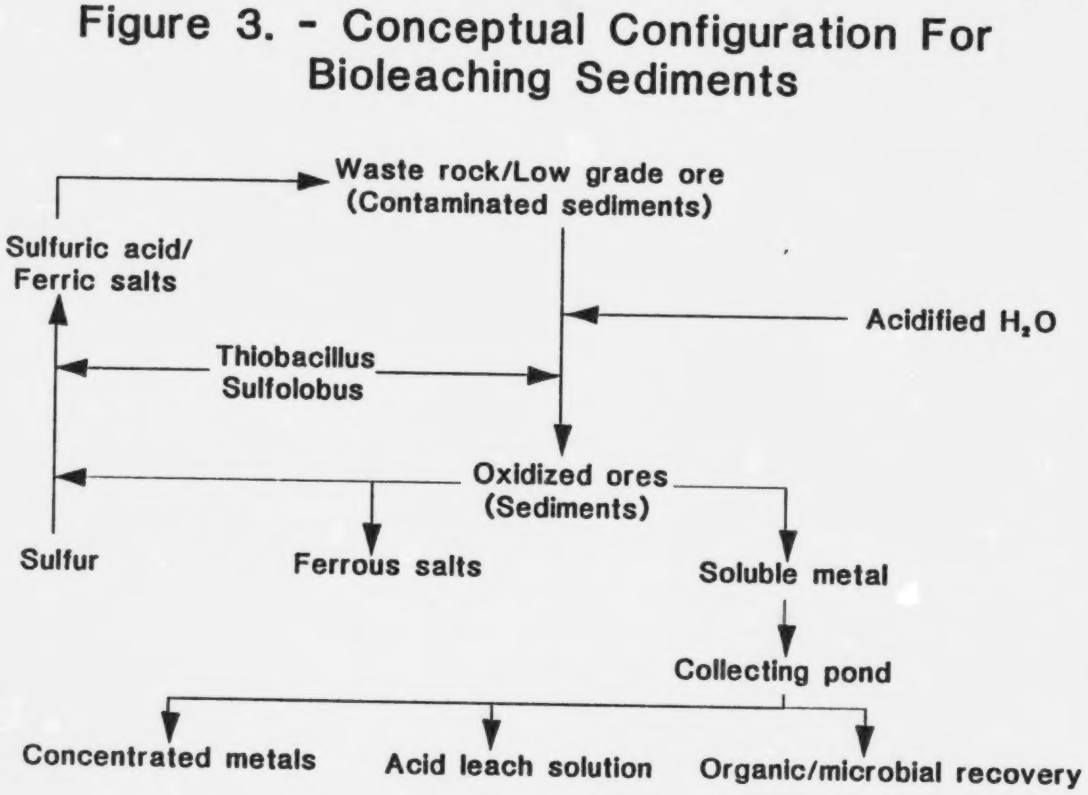
Figure 3. - Conceptual Configuration For Bioleaching Sediments
Waste rock/Low grade ore (Contaminated sediments)
Sulfuric acid/ Ferric salts
Acidified H_2O
Thiobacillus Sulfolobus
Oxidized ores (Sediments)
Sulfur
Ferrous salts
Soluble metal
Collecting pond
Concentrated metals
Acid leach solution
Organic/microbial recovery

Figure 6.1.3. Conceptual configuration for bioleaching sediments.

6.2 Biological Treatment of Metal-Contaminated Water

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Acid coal mine drainage

Acid mine drainage (AMD) is a common water pollution problem on active and abandoned coal mine sites in the eastern United States. AMD forms when surface mining brings unweathered pyrite-containing rocks to the surface or when deep mining allows oxygen to contact buried pyritic strata. In the absence of neutralizing compounds, the drainage that results can be extremely acidic and contaminated with dissolved iron, manganese, and sulfate. Drainages with pH < 3.0 and concentrations of sulfate greater than 1,000 mg/L, iron greater than 50 mg/L, and manganese greater than 10 mg/L are common. Where water flows through alkaline materials (such as limestone) before surfacing, the drainage is less acidic and occasionally circumneutral, but it can still contain high concentrations of sulfate and metals.

Current water quality standards in the United States require that mine discharges have a pH between 6 and 9, total iron concentration less than 3.0 mg/L, and manganese less than 2.0 mg/L. At thousands of active and inactive mine sites, drainage does not meet these standards and is being treated before discharge by the mining company. At thousands of other sites that were abandoned prior to the enactment of water pollution laws or were operated by companies that have gone bankrupt, untreated AMD is polluting receiving water systems.

The standard mine drainage treatment system involves the addition of alkaline chemicals to the water, which raises the pH and causes metals to precipitate in a settling pond. These systems are expensive, often costing tens or hundreds of thousands of dollars per year for chemicals, operation, maintenance, and disposal of the metal-laden sludge. Because the drainage on many sites will likely be contaminated for decades, there is financial incentive to find alternative water treatment systems.

The constructed wetland concept has its roots in observations of natural *Sphagnum* peat wetlands that received acid mine drainage and, instead of being adversely affected, appeared to clean the polluted water. These observations instigated the idea that wetland systems might be used for the intentional treatment of mine drainage. Because the discharge of AMD into a natural wetland is prohibited by several laws, it has been necessary to construct wetlands that act solely as water treatment systems.

Initially, most wetland research and construction efforts mimicked the original observations by using *Sphagnum* moss and peat. Despite promising lab results, virtually all field tests of *Sphagnum*-dominated constructed wetlands failed to provide sufficient water treatment for more than several months. *Sphagnum* proved quite sensitive to the stresses associated with transplanting, abrupt changes in water chemistry, excessive or insufficient water depth, and excessive accumulation of iron. At most sites, the moss died within the first growing season.

Today, almost all wetlands constructed to treat AMD are planted with *Typha latifolia*, common cattails. *Typha* is readily available to most sites, transplants well, and has proved tolerant of a wide range of water conditions. Occasionally, *Scirpus* spp. (bulrushes) and *Equisetum* spp. (horsetails) are also planted, but even these wetlands are generally dominated by cattails after the first few years of system operation.

Most constructed wetlands include 15-45 cm of an organic substrate in which the emergent plants can root. Topsoil, rotten animal manure, spoiled hay, and compost have been used. In western Pennsylvania, mushroom compost, a waste product of mushroom farming, has become a widely used organic substrate. At sites with acidic drainage, 8-16 cm of crushed limestone is often spread underneath the organic substrate to provide some neutralization.

Constructed wetland systems usually consist of a series of shallow pits or cells. This design makes flow control much easier than with a single, large wetland. The cells are filled with substrate, planted with *Typha*, and flooded with mine drainage. In most systems, water depth is 5-15 cm above the substrate and flow is quite slow. Hay bales and logs are sometimes used as barriers to enhance serpentine flow pattern, prevent channelization, and increase the contact of AMD with the wetland substrate and vegetation.

AMD is now being treated biologically in constructed wetlands at over 300 mine sites in the bituminous coal region of the eastern United States. In general, the processes at work in these systems are aerobic. The oxidation of ferrous iron to ferric iron and the subsequent precipitation of iron oxyhydroxide floc, for example, are dominant processes:

$$Fe^{2+} + 0.25O_2 + 1.5H_2O \dashrightarrow FeOOH\ (solid) + 2H^+ \quad (1)$$

Ferrous iron tends to autooxidize in aerated solutions at pH values greater than 6, while in more acidic water naturally- occurring bacteria catalyze the reaction. Although iron oxidation and hydrolysis processes are effective at removing much of the iron from the AMD, these processes do nothing to help raise the pH of the water or lower the acidity. In fact, the pH of water can be lowered by these reactions (equation 1). Many constructed wetlands with circumneutral pH and iron-contaminated inflow water actually produce water with a lower pH.

Ironically, bacterial processes capable of increasing the pH and alkalinity of AMD entering constructed wetlands are already found there, but current wetland designs do not take advantage of them. Probably the most useful of these processes for treating AMD is bacterial sulfate reduction, a naturally-occurring reaction that proceeds in many environments in the absence of oxygen and in the presence of suitable organic substrates and sulfate. Sulfate-reducing bacteria use organic carbon and sulfate in the process of anaerobic respiration:

$$2CH_2O + SO_4^{2-} \dashrightarrow H_2S + 2HCO_3^- \quad (2)$$

The reaction has promise in the treatment of acid- and metal- contaminated mine waters because the by-products of the reaction, hydrogen sulfide and bicarbonate, can precipitate many metals and raise the pH of the water, respectively.

Sulfate reduction rates have been measured in the sediments of marine and freshwater environments. Sulfate reduction rates often vary over several orders of magnitude at any given location due to the heterogeneous nature of sediments. Measured rates range from approximately 0.4 to 3000 nmol cm^{-3} day^{-1}. Oxygen, low temperatures, low concentrations of organic matter and sulfate, and low pH all tend to limit sulfate reduction rates. Recent work at the Bureau of Mines has established that sulfate reduction does occur in constructed wetlands and can play a significant role in the treatment of AMD. Water quality data from several constructed wetlands demonstrating the influence of both aerobic and anaerobic treatment processes will be shown.

Metal mine drainage

Recently, the U.S. Bureau of Mines has begun to exploit the bacterial sulfate reduction process studied in wetlands for the treatment of mine waters contaminated with metals other than iron and manganese. Many heavy metals, such as Cd, Cu, Pb, Hg, Ni, Ag, and Zn, can be precipitated as insoluble sulfides in the presence of sufficient hydrogen sulfide. Although little evidence has been accumulated to date, it seems unlikely that wetland systems will be a satisfactory way to treat these metals due to the likelihood of their bioaccumulation in plants and animals. Research efforts have therefore been directed towards the development of contained sulfate reduction systems consisting of barrels or tanks with sufficient organic matter to enhance anaerobic bacterial activity. Laboratory experiments have been performed and pilot-scale studies are currently underway at several locations, including the U.S. Bureau of

Mines research mine in Pittsburgh, PA, and at a zinc smelter Superfund site near Palmerton, PA. The results of this work will be discussed and the potential use of wetland and bacterial sulfate reduction systems in the bioremediation of contaminated sediments will be addressed.

6.3 Bioleaching of Ores

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INTRODUCTION

Metal Biosolubilization in Nature

The most important biogeochemical roles that microbes play in nature have to do with the transformation and cycling of elements, such as carbon, oxygen, nitrogen, sulfur, and phosphorus. But metals such as iron, manganese, calcium, potassium, mercury, selenium, and zinc are also transformed in nature. The various chemical reactions in these cycles are beneficial, and often essential, to make the minerals available to indigenous flora in soluble form for their metabolism.

But natural, or uncontrolled, biosolubilization can create environmental problems. For instance, oxidation of pyritic minerals by native microorganisms, such as *Thiobacillus ferrooxidans* or *Thiobacillus thiooxidans* can lead to serious water pollution problems in coal mining regions. *Thiobacilli* are chemolithoautorophs, which obtain their energy by oxidizing reduced iron and sulfur moieties and their carbon from CO_2 in the air. Sulfuric acid produced by the action of the bacteria on sulfide minerals present in the coal is responsible for solubilization of metal ions which contaminate the mine waters. Acid mine drainage is also a problem in metal mines in the west, especially lead and zinc districts, where the sulfidic ores are attacked by similar microorganisms.

Metal Bioleaching from Ores by *Thiobacillus* Bacteria

Naturally occurring *Thiobacillus* bacteria play a significant role in leaching of copper from heaps and dumps of low-grade ore (*1*). The microbes can oxidize reduced copper sulfide minerals by a direct mechanism to produce soluble cupric sulfate, which is concentrated and recovered as copper metal. But an even more important mechanism is the indirect oxidation of copper sulfides by ferric iron formed by direct attack of the bacteria on iron pyrite which is also present in the ore:

DIRECT LEACHING (direct attack of mineral by microbes)

$$4\ FeS_2 + 15\ O_2 + 2\ H_2O \xrightarrow{\text{bacteria}} 2\ Fe_2(SO_4)_3 + 2\ H_2SO_4$$

INDIRECT LEACHING (attack by biologically generated ferric iron)

$$CuFeS_2 + 2\ Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5\ FeSO_4 + 2\ S$$

The leachant is regenerated by further biooxidation:

BACTERIAL LEACHANT REGENERATION

$$2\ S + 3\ O_2 + 2\ H_2O \xrightarrow{\text{bacteria}} 2\ H_2SO_4$$

$$4\ FeSO_4 + O_2 + 2\ H_2SO_4 \xrightarrow{\text{bacteria}} 2\ Fe_2(SO_4)_3 + 2\ H_2O$$

By similar mechanisms, direct and indirect, *Thiobacillus ferrooxidans* is known to aid in the removal of uranium, zinc, cobalt, nickel, cadmium and other metals from sulfidic ores.

Metal Bioleaching from Ores by Heterotrophic Microorganisms

Heterotrophic microorganisms, those which require organic carbon for their growth and energy needs, can also solubilize metals from rocks and minerals. Heterotrophs have been shown to be capable of extracting nickel and aluminum from silicate ores, waste products and clays (*2,3*) and bacterially mediated, reductive dissolution of iron- and manganese-bearing ores has also been demonstrated (*4-6*). Heterotrophs are also known to be capable of breaking down silicate minerals, the principal component of most rocks, and can utilize and remove phosphorus from phosphate ores.

Like the chemolithoautotrophs, heterotrophic microorganisms also employ direct and indirect leaching mechanisms. Direct leaching utilizes reductive solubilization of metal species as a form of respiration. Or the microorganisms can indirectly produce an acid, base, or ligand the solubilized metals.

Bacterial Pretreatment of Ores by *Thiobacillus Bacteria*

Biooxidation is increasingly being implemented as a pretreatment step in the processing of refractory precious metal ores (*7*). For instance, gold which is locked inside iron sulfide minerals often cannot be extracted by conventional cyanidation. But, ores of this type can be pretreated with sulfur oxidizing microorganisms, most commonly *Thiobacillus ferrooxidans*, which break open the pyrite matrix and allow access to the gold by cyanide during subsequent processing. A 1500 ton per day biooxidation plant has recently been built in Central Nevada, and smaller plants have been operated worldwide.

Bacterial pretreatment has also been extensively investigated as a means of removing pyritic sulfur from coal.

BIOLEACHING TO EXTRACT METALS FROM ORE MATERIALS

The Bureau of Mines has developed a research group at the Reno Research Center which has been conducting biohydrometallurgical research for the past several years. The focus of this work has been the biosolubilization of manganese from low-grade domestic ores by heterotrophic microorganisms, and the biooxidative pretreatment of a sulfide concentrate containing platinum-group metals using *Thiobacillus ferrooxidans* bacteria. The research has taken primarily an applications approach, with the more basic work on mechanisms and physiology of the microbes being undertaken via contract research at the Idaho National Engineering Laboratory.

Bioleaching of Manganese Ores

Because manganese is a low-value commodity, this work is directed at low-cost mining and processing technology - open pit mining, and heap leaching. The microorganisms utilized are native to the ores or are introduced via the nutrient used to feed them (molasses). In the laboratory, bioleaching of manganese ores is being investigated on three different scales:

(1) shake-flask tests - conducted as screening experiments to quickly obtain information on the ability of finely ground ore to be leached under various conditions. These experiments can be kept sterile, if desired, to test for chemical leaching effects or to run controls.

(2) column tests - utilize information obtained during flask tests. The columns allow for some control of the biological system and employ larger-sized material. Nutrient medium is recirculated through the ore bed and is replaced when depleted.

(3) open, non-sterile simulated heaps - allows for contamination of the bioleach system by

air-borne microorganisms, especially mold spores, which are present in the environment surrounding the heap. These experiments simulate conditions likely to be incurred in a real-world heap leaching situation.

Shake-flask tests:

Several nutrient media have been evaluated and the best results have been obtained by using molasses to feed the microorganisms. The experiments are conducted by slurrying ground ore with diluted (5 wt pct) molasses and sampling the flasks weekly so that the manganese content of the solutions can be monitored. The results of shake-flask screening tests using food-grade molasses are shown in Table 6.3.1. Several oxide ores were found to be readily amenable to bioleaching, with extractions of more than 95 pct being attained in 4 weeks. But only 27 pct of the manganese was extracted from the sulfidic Black Cloud ore. This ore would best be treated by sulfide oxidizing bacteria such as *Thiobacillus ferrooxidans*.

The antimicrobial agent sodium azide was added to some experiments to eliminate biological activity and to allow determination of the extent of chemical leaching by the molasses. These control tests showed that chemical leaching by the 5 pct molasses solution varied from 2 to 13 pct for the ores tested.

Factory molasses, which is the residual in food-grade molasses production, was also evaluated. This by-product is sold for animal feed for approximately $0.05/lb and could provide a low cost nutrient source for bioleaching. Shake-flask bioleaching tests were conducted using factory molasses in the same manner as the tests with food-grade molasses. Results for bioleaching Three Kids ore with 5 pct factory molasses are shown in Figure 6.3.1. The results were similar to those obtained using food-grade molasses -- 97 pct of the manganese was extracted in 7 weeks.

When slurries of unsterilized ore and molasses medium were inoculated with bacteria from previous experiments, only 70 pct of the manganese was extracted in the first 5 weeks, and some precipitation of the manganese occurred during continued leaching. Precipitation occurs when the organic carbon in the medium has been depleted and the pH rises. It appears that the inoculum probably contained microorganisms which competed with the manganese solubilizing microbes for the carbon in the medium.

The aerobic dissimilation of carbohydrates, such as glucose, involves a series of enzymatic changes, which may be divided into two parts: an initial breakdown to pyruvic acid and the subsequent oxidation of pyruvate to CO_2 and H_2O, via the tricarboxylic acid, or Krebs, cycle. Other pathways for pyruvate utilization also occur, depending on the microorganism and the conditions. During the Krebs cycle, pyruvate is converted to citric acid, which undergoes a series of enzymatic oxidations, decarboxylations, and transformations, forming (in succession) isocitric, α-ketoglutaric, succinic, fumaric, malic, and oxaloacetic acids. The net result of this cyclic process is the complete oxidation of one molecule of acetate to CO_2 and H_2O with each turn of the cycle.

To evaluate whether Krebs cycle acids and other organic acids which can be products of respiration or fermentation pathways are capable of solubilizing manganese from its ores, a series of shake-flask tests was performed in which Three Kids ore was leached abiotically with a number of organic acids. The tests were conducted under the same conditions as bioleach experiments and the carbon content of each leach liquor was 4 g/L. Results are tabulated in Table 6.3.2. The acids which extracted the most manganese were L-malic (91 pct), α-ketoglutaric (91 pct), and citric (84 pct). Leaching rate was fastest with citric acid, the first acid formed during the Krebs cycle. These experiments suggest that an indirect leaching mechanism, i.e., leaching by organic acids produced during carbohydrate metabolism, may be responsible for the removal of manganese from Three Kids ore. Examination of solution potential and pH data indicate that leaching is not the result of acid generation, but probably involves reduction of the higher oxides of manganese to soluble manganous ions by the organic compounds, possibly accompanied by chelation of the dissolved metal species.

These tests do not rule out a direct leaching mechanism. Other researchers have shown that both indirect and direct leaching mechanisms are operative in manganese bioleaching systems.

Column and heap bioleaching tests:

Column and simulated heap leaching experiments are being conducted by recycling medium through beds of Three Kids ore. Size of the tests has varied from 400 grams of ore in 5-cm i.d. columns and 2 L of medium up to 34 kg of ore in a 32 cm i.d. open cylindrical tank and 20 L of medium. Occasional replenishment of the medium is required to keep the bioleaching going. A typical column bioleaching curve is shown in Figure 6.3.2. Similar curves are generated during heap bioleaching. Manganese dissolution increases until the medium is spent and if the test is allowed to proceed without replenishment of the nutrient, the pH of the system increases and manganese precipitates from solution. As long as sufficient nutrient is present, the pH remains on the acid side of neutral (5.5 to 7) and manganese stays in solution. We have operated some columns for as long as a year and manganese has continued to leach from the ore.

Best results to date have been attained using molasses to bioleach minus-1/4 in. ore in 5 cm columns (Table 6.3.3). For instance, 70 pct manganese extraction was obtained in 29 weeks in a test using 3 pct molasses, and 30 pct extraction has been obtained after 6 weeks in an ongoing column which is being leached with 5 pct molasses. A control test, in which sodium azide was added to the medium, showed only 5 pct extraction in 6 weeks and visible evidence of growth was negligible compared to active bioleach columns. The data also show that minus-3/4 in. ore leaches much more slowly than the 1/4 in. material, which is not unexpected. We are not sure whether the ore particles are being wetted through to the core.

We have isolated various microorganisms from bioleach solutions and identified them by fatty acid analysis and by using a standard biochemical multitest system. Because molasses caramelizes at sterilization temperatures, the microbes were cultured on tryptic soy agar (TSA) plates. This technique may not be definitive, because it is possible that the TSA selects for microorganisms that do not dominate in the molasses-based leach medium. At this point, we have not determined which species are responsible for the leaching. We also do not know whether the manganese solubilizing microorganisms come from the molasses, or from the ore itself. To answer these questions, we recently hired a person to undertake the microbiological aspects of this project. We hope to have some results in the next few months.

Biooxidation of Platinum and Gold Ores

As stated earlier, sulfide ores are often refractory, i.e. resistant to conventional cyanidation, because the sulfide minerals encapsulate the precious metals and prevent access of the leachant to the insides of the mineral particles. To overcome this problem, refractory gold ores are treated by pressure oxidation or by roasting prior to cyanidation. Pressure oxidation results in high capital costs, and roasting produces SO_2 which requires off-gas treatment. Biooxidation is another pretreatment option which has been gaining increased attention in recent years. In fact, a 1500 tpd biooxidation plant came on-line earlier this year near Austin, NV. Smaller plants are in operation worldwide, and the technology is being marketed by a number of companies.

The Bureau of Mines has been investigating biooxidation as a possible environmentally acceptable alternative to smelting for pretreating a sulfide platinum-group metal concentrate from the Stillwater Complex in Montana. Smelting is a high-temperature processing step which, like roasting, evolves SO_2, and requires strict environmental controls. The Stillwater Complex holds the only PGM deposit in the United States and this research is being conducted as part of the Bureau's strategic minerals program. A mineralogical description of the concentrate is shown in Table 6.3.4.

Biooxidation is being conducted at 30° C in stirred, batch reactors up to 5 liters in size. Normally a 10 pct pulp density is maintained. *Thiobacillus ferrooxidans* bacteria are fed with a simplified mineral salts nutrient medium composed of three salts dissolved in water [$(NH_4)_2SO_4$, KH_2PO_4, $MgSO_4$]. Ferrous sulfate is sometimes added to the medium to give the bacteria a ready supply of energy and to support rapid growth during the early stages of the process. We have been trying to wean the bacteria from ferrous iron by gradually decreasing the amount added to the medium. The intent is to force the microbes to obtain their energy by oxidizing the sulfides present in the concentrate. The laboratory reactors are aerated with a mixture of air containing 5% CO_2. The dissolved oxygen content and oxygen transfer rates are important factors in biooxidation reactors, and *Thiobacillus ferrooxidans* obtains its cellular carbon from the CO_2. We have found that operating the reactors in a draw and fill mode, i.e., periodic settling, decantation, and replacement of the medium, enhances sulfide oxidation. Table 6.3.5 shows the effects of medium replacement and use of aeration. Both factors enhanced the amount of sulfide oxidation achieved.

Using draw and fill operation, we have been able to oxidize up to 94 pct of the sulfide in the Stillwater concentrate over a period of 5 weeks. Biooxidation destroys the pentlandite, pyrite, and chalcopyrite minerals in the concentrate, and leaches the nickel and some of the copper. The PGM remain in the residue, primarily as sulfide minerals, even though most of the sulfur has been removed. Mineralogical characterization with the scanning electron microscope showed the following relative preponderance of PGM minerals in the biooxidized residue:

$$PdS > (Pt,Pd,Ni)S >> PdTe = PtTe_2 > PtFe = PtS.$$

Chemical Leaching of Biooxidation Residue

After biological pretreatment, the solids are treated chemically to extract the precious metals. We have investigated several leachants: (1) oxidative chloride (aqua regia and H_2O_2-HCl), the traditional method for extracting PGM from minerals; (2) thiourea, a known gold extractant which operates in the acid pH range; and (3) cyanidation, which is conducted at high pH.

Highest extractions to date have been obtained with cyanidation at 80° C. Results are shown in Table 6.3.6. Palladium, rhodium and gold extractions are quite reasonable, but the highest platinum extraction obtained so far is only 34 pct. SEM examination showed that the palladium-bearing minerals in the residue had decreased considerably, but the platinum-bearing minerals still remained:

$$(Pt,Pd,Ni)S > PtS >> PdS >> PtTe_2 > PtFe.$$

The poor leaching of platinum may be a kinetic problem, or it may be the result of electrochemical (galvanic) effects. Selective metal leaching by galvanic effects is based on the fact that physical contact between dissimilar metal sulfides immersed in dilute sulfuric acid/ferric sulfate solution will create a galvanic cell. The sulfide mineral with the highest rest potential will become cathodically protected (passivated), while ones with lower rest potentials (anode) will be leached. The Stillwater concentrate is a multimetal sulfide mixture and the rest potentials of the principal base metal sulfide minerals fall into the following order:

pyrite > chalcopyrite > pentlandite > pyrrhotite.

Pyrrhotite should leach first, pyrite last. Destruction of the pentlandite should liberate the contained palladium, and we do see good palladium removal during cyanidation. Because platinum sulfides are very insoluble and stable, their rest potentials are expected to be higher than that of pyrite. As a result, they should be even harder to leach. It may be necessary to have almost complete oxidation of the sulfide minerals before high platinum extractions are possible.

We have recently set up a prototype continuous stirred tank bioreactor system to see if we can improve sulfide oxidation, in hopes of improving the recovery, especially platinum in the second stage chemical leach. If that doesn't work, we will be looking at different sulfide oxidizing microorganisms such as the thermophile *Sulfolobus* to see if more efficient biooxidation can be attained.

SUMMARY

Natural biosolubilization of metals can be beneficial, in that it provides minerals to indigenous flora for their metabolic needs, and it can create environmental problems, such as acid mine drainage. Controlled biosolubilization of metals from ores, biooxidation and bioleaching, are showing increased importance as alternatives to conventional chemical oriented procedures for recovering metals from ores.

The bioleaching research group at Bureau of Mines Reno Research Center has been investigating biological treatment of ores and mineral concentrates for the past several years. Emphasis has focussed on the biosolubilization of manganese from its ores using heterotrophic microorganisms and the biooxidation of a sulfidic platinum-group metal concentrate with the acidophilic chemolithotroph *Thiobacillus ferrooxidans*. The research has shown that biological treatment can be used to extract metals from ores and that biological treatment can also be used to make minerals more amenable to subsequent chemical treatment to remove metal values.

Sediments and ores are both rock-based substances. The fact that metals can be removed from ores by biological action indicates that there is good potential that biological extraction of metals from sediments can be successful.

REFERENCES

1. Hiskey, J. B. and R. Bhappu (1987). Role of Oxygen in Dump Leaching. **In:** *Proc. of Internatl. Sympos. on the Impact of Oxygen on the Productivity of Non-Ferrous Metallurgical Processing*, G. Kachaniwsky and C. Newman (eds.), Pergamon Press, pp. 165-182.

2. Bosecker, K. (1989). Bioleaching of Valuable Metals From Silicate Ores and Silicate Waste Products. **In:** *Biohydrometallurgy*, J. Salley, R. G. L. McCready, and P. L. Wichlaez (eds.), Canmet SP89-10, pp. 15-24.

3. Groudev, S. N., and V. I. Groudeva (1986). Biological Leaching of Alumina from Clays. **In:** *Workshop on Biotechnology for the Mining, Metal-Refining, and Fossil Fuel Processing Industries*, H. L. Ehrlich and D.S. Holmes (eds.), John Wiley And Sons, N. Y., pp. 91-99.

4. Ehrlich, H. (1980). *Bacterial Leaching of Manganese Ores, Biochemistry of Ancient and Modern Environments*, Springer-Verlag, Berlin, pp. 609-614.

5. Yopps, D. L., E. G Noble, E. G. Baglin, and J. A. Eisele (1989). Bioleaching of Manganese Ores (Poster). *Biohydrometallurgy 1989*, Jackson Hole, WY, Aug. 13 - 18, 1989.

6. Holden, P. J., and J. C. Madgwick (1983). Mixed Culture Bacterial Leaching of Manganese Dioxide. *Proc. Australas. Inst. Min. Metall.*, **286:** 61-63.

7. Hutchins, S. R., J. A. Brierley, and C. L. Brierley, *Microbial Pretreatment of Refractory Sulfide and Carbonaceous Ores*, 116th Annual AIME Meeting, Feb 23 - 27, 1987, SME Preprint 87-143, 1987, 16 pp.

Table 6.3.1. Shake-Flask Bioleaching of Manganese Ores.

Source	Mineralogy	Mn Content, pct	Mn Extraction, pct	
			Bioleach	Azide Control
Three Kids, Nevada	Mn oxide quartz feldspar	15.6	79	12
Silver Cliff, Colorado	Mn oxide quartz potassium feldspar	3.7	97	13
Algoma-Zeno, Minnesota	Mn oxide Fe_2O_3 quartz	15.5	98	13
Black Cloud, Colorado	Mn carbonate Fe-Pb-Zn sulfides	0.5	27	4

Conditions: 2 g minus 48-mesh ore, 100 mL of 5 wt-pct food-grade molasses medium, 4 weeks, ambient temperature, 200 RPM

Table 6.3.2. Abiotic Leaching of Three Kids Ore with Organic Acids.

Acid	Mn Extraction, pct
L-Malic	91
α-Ketoglutaric	91
Citric	84
Formic	65
Lactic	44
Oxalic	11
Succinic	6
Fumaric	4
Acetic	4

Conditions: 2 g minus 48-mesh ore, 100 mL of solution containing 4 g/L organic carbon, 2 weeks, ambient temperature, 200 RPM

Table 6.3.3. Column and Heap Bioleaching of Three Kids Ore.

Weight Ore, kg	Ore Size, in	Column i.d., cm	Medium Type	Medium Vol, L	Mn Extn, pct	Leach Time, wks
0.4	-1/4	5	1GA	2	28	52
0.5	-1/4	5	3FGM	5	70	29
0.5	-1/4	5	5FGM	5	30	6
0.5	-1/4	5	5FGM/ azide	5	5	6
9	-3/4	12.7	3FGM	20	2	5
34	-3/4	32	3FGM	20	1	15
36	-3/4	heap	1GA	20	2	37

Medium:
1GA .....1 wt-pct glucose, $(NH_4)_2SO_4$
3GFM....3 wt-pct food grade molasses
5FGM....5 wt-pct food grade molasses

Table 6.3.4. Stillwater Ore Minerals.

BASE METALS	pyrite, chalcopyrite, pentlandite
PLATINOID MINERALS	PGM sulfides Pt-Fe alloy solid solution in pentlandite (Pd)
GANGUE	Al, Ca, Fe, Mg silicates

Table 6.3.5. Bio-oxidation of Stillwater Flotation Concentrate.

Medium Replacement	Air/CO2 Sparge	Sulfide in Solids, pct Week 1	2	3	4	5	Total Sulfide Oxidized, pct.
No	No	ND	ND	6.1			0
No	Yes	5.6	4.2	1.6	1.4	1.1	83
Yes	No	4.0	3.5	ND	ND	3.8	38
Yes	Yes	5.2	2.3	ND	0.9	0.3	94

Conditions: 300 g concentrate, 6.1 pct sulfide, 3 L ATCC medium 64, 300 mL inoculum of 72-hr *T. ferrooxidans* A-6 culture.

Table 6.3.6. Cyanidation of Bioleached and As-Received Stillwater Concentrate.

pct sulfide oxidized during bioleaching	Extraction, pct Pt	Pd	Rh	Au
94	35	76	90	99
79	24	73	92	98
70	20	74	79	98
60	22	76	91	--
0 (as received)	16	64	43	97

Conditions: 30 g concentrate in 600 mL of 1 pct or 2 pct CN solution, 80° C, 23 h.
Head Analysis: 10 oz/t Pt, 33 oz/t Pd, 0.3 oz/t Rh, 0.5 oz/t Au, 6.1 % S.

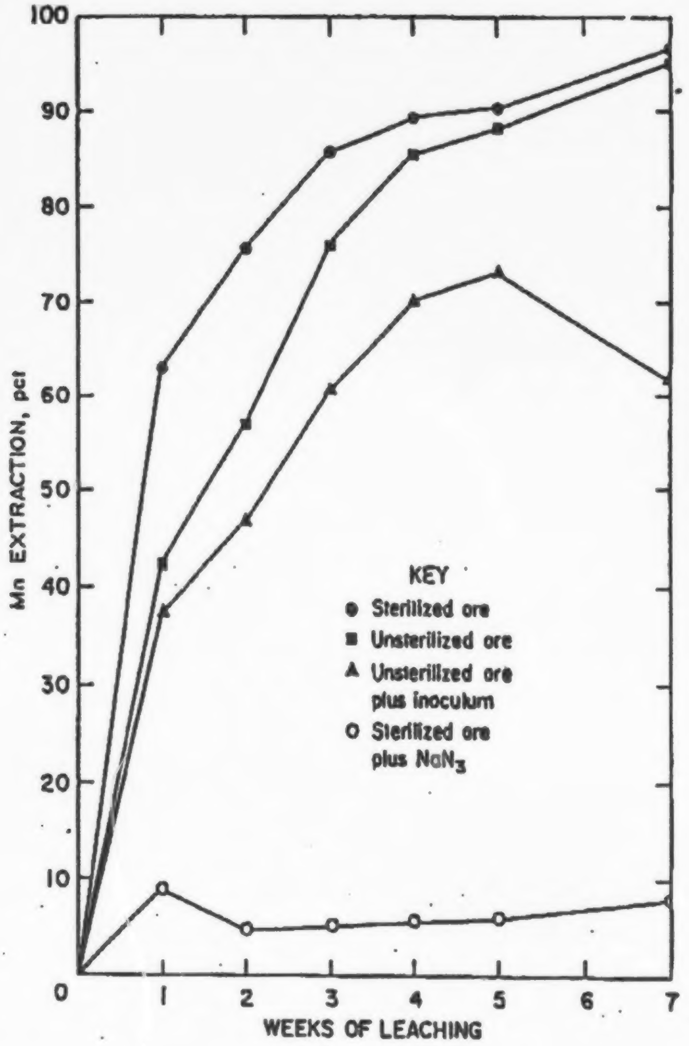

Figure 6.3.1. Shake-flask bioleaching of Three Kids ore, 5 pct. factory molasses.

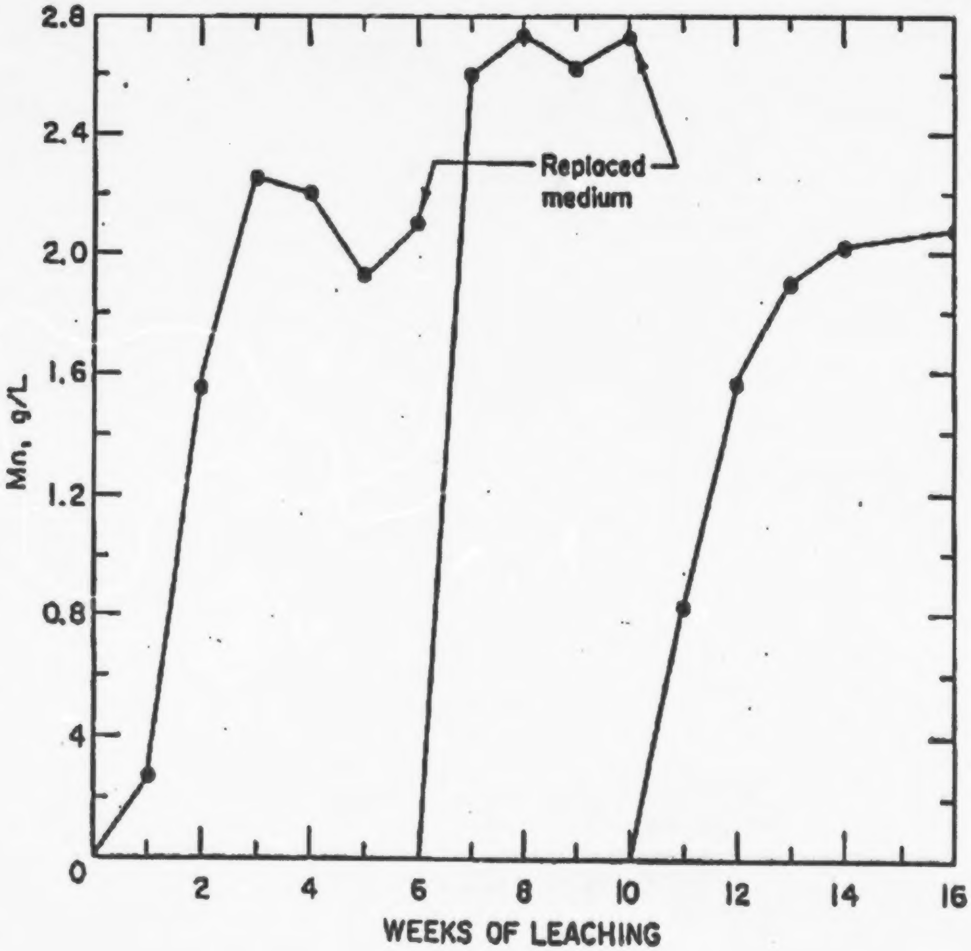

Figure 6.3.2. Column bioleaching of Three Kids ore, 3 pct. food-grade molasses.

6.4 Mechanisms of Bacterial Metals Removal From Solids

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Abstract

The Great Lakes area sediments are contaminated with varying amounts of heavy metals and polychlorinated organic matter. With respect to the bioremediation of metallic contents of these sediments, it was shown that a number of microorganisms exist which can effectively solubilize heavy metals. The basic reaction mechanisms of bioleaching processes were discussed and the effects of semiconductor character of the sulfide substrate explained. A special emphasis was made to comment on INEL's bioremediation capability.

Introduction to Sediment Environments

Sediments are commonly defined as solid material that has settled down from a state of suspension in a liquid (*1*). The sediments of marine origin are divided into three main classes (*1,2*): detrital material derived from the erosion of the continents, biogenic material that is formed by biological productivity, and autogenic material that is formed *in situ*.

Detrital material consists mostly of alumino-silicates. Biogenic components are produced from plankton tissues, algal mats, and other microbial organisms (*4*) in the surface waters of lakes, estuaries and seas, and contain calcites as well as organic matter. Autogenic materials consist of mineral phases (sulfides, phosphates, and carbonates). Biogenic sediments are most active in the upper part of the sediments and it is very rare to find biological processes below a depth of half a meter (*3*). When a phosphate-rich wastewater is introduced into very hard lake water (containing high calcium concentration) the following reactions may occur (*5*):

[1] $5\ Ca^{+2} + OH^{-} + 3\ PO_4^{-3} \longrightarrow [Ca_5(OH)(PO_4)_3]$ hydroxyapatite

Near the surface where CO_2 concentration is relatively nigh, calcium carbonate is formed:

[2] $Ca^{+2} + 2\ HCO_3^{-} \longrightarrow [CaCO_3] + CO_2 + H_2O$

When the pH is locally raised by photosynthetic reaction it yields;

[3] $Ca^{+2} + 2\ HCO_3^{-} + hv \longrightarrow CH_2O + [CaCO_3] + 0_2$

A decrease in pH can result in the production of insoluble humic-acid-base metal sediments (*6*). Introduction of acidic mine tailings and industrial drainages into rivers and lakes results in considerable heavy metal contamination and formation of toxic inorganic sediments. Biological activity is responsible for the formation of sulfide-bearing heavy metal sediments. For example, there are a number of sulfate reducing bacteria (*7*), which perform an anaerobic respiration by using sulfate as final electron acceptor and by oxidizing organic compounds (*8*); for example:

[4] $\underset{\text{(lactate)}}{2\ CH_3\text{-}CHOH\text{-}COONa} + H_2SO_4 \xrightarrow{\text{bacteria}} H_2S + 2\ CH_3\text{-}COONa + 2\ CO_2 + H_2O$

The resulted H_2S will react with the heavy metal ions in solution:

[5] $M^{2+} + H_2S \longrightarrow (MS) + 2\ H^+$

where M^{2+} is Fe^{2+}, Mn^{2+}, As^{2+}, Zn^{2+}, Cd^{2+}, etc. The metal sulfide precipitate (MS) will be contained in the sulfide autogenic sediment. The sulfate reduction reactions will especially be predominant during the winter season when the surface of the lake is covered by ice and snow and infiltration of oxygen into the lake water is considerably limited. The sediments may vary from completely *oxic*, where the supply of organic carbon is less than the supply of oxygen, to wholly *anoxic*, where the supply of organic carbon is much greater than the supply of oxygen. The changing chemistry of the sediments is reflected in changes in redox potential and the pH. The redox system was used for the classification of the chemical sediments (*2,9*) already in the 1940s and 1950s.

Great Lake Sediments

The sediments from the Great Lakes areas (Saginaw Bay, Michigan; Sheboygan Harbor, Wisconsin; Grand Calumet River, Indiana; Ashtabula River, Ohio; and Buffalo River, New York) are known to be highly toxic (*10*). The remedial action plans include 42 areas of the Great Lakes where the sediments contain varying amounts of heavy metals (arsenic, cadmium, chromium, copper, iron, lead, manganese mercury, nickel, silver, and zinc) as well as organic matter (polychlorinated biphenyls, polyaromatic hydrocarbons, oils, greases, and cyanides). Thus, the sediment environment of these areas is a very complex material, and it is likely that there is more variability in the sedimentary system than uniformity. Therefore, it can be anticipated that a simple approach for the remediation of all Great Lakes areas may not be feasible and methods based upon site specific information must be worked out. In this context, the bioremediation of contaminated sediments presents an alternative to the chemical and physical remediation possibilities. This paper will report on the background information on possible bioremediation of inorganic (metallic) contents of the Great Lakes Sediments and point out INEL's capabilities.

Microorganisms

It is known and well documented that microorganisms are playing an important role in the formation and solubilization of mineral deposits since geological time (*11, 12*). Since the metal contents of the Great Lakes area sediments are occurring probably in forms of sulfides, oxides, silicates and carbonates, it is likely that they can be extracted especially by the iron and sulfur oxidizing thiobacilli, which are know as the leaching microorganisms. The most frequently studied bacterium is called *Thiobacillus ferrooxidans* (*13*). It oxidizes ferrous iron and reduced-valence inorganic sulfur compounds (*14*):

[6] $2\ FeSO_4 + H_2SO_4 + 0.5\ O_2 \xrightarrow{bacteria} Fe_2(SO_4)_3 + H_2O$

[7] $MS + 2\ O_2 \xrightarrow{bacteria} MSO_4$

where M is a bivalent heavy metal. The metal sulfides, MS, are generally insoluble in the acidic nutrient media, while their corresponding sulfates are soluble. Hence is the dissolution process. Other leaching bacteria are (*15,16*) *Thiobacillus thiooxidans* which oxidizes elemental sulfur and thiosulfate but not metal sulfides; *Leptospirillium ferrooxidans* which oxidize ferrous iron and pyrite; *Thiobacillus organoporus* oxidizes elemental sulfur and a number of metal sulfides (PbS, Bi_2S_3, Sb_2S_3, ZnS) thermophilic bacteria which are active between 45 and 85°C (*Sulfobacillus thermosulfiodooxidans, Sulfolobus acidocaldarius, Sulfolobus brierley, Sulfolobus solfataricus*) can oxidize metal sulfides, ferrous iron and elemental sulfur. Some of the thermophilic species require the presence of minute amounts of yeast extracts. In addition to the above lithotrophic bacteria, there are a number of heterotrophs present in the naturally occurring heap and dump leach media (*17*). However their specific contribution to the solubilization of metal sulfides is not well understood and documented.

Leaching Mechanisms

Metals can be extracted from insoluble minerals (sulfides, oxides, carbonates, etc.) directly by metabolic action of microorganisms or indirectly by the product of their metabolism (*18*). The biological reactions relevant for removal of toxic metal contents from sediments are primarily oxidation reactions of metal sulfides, such as of iron pyrite in which sulfur is in the -2 oxidation state (*19*), that will be oxidized to ferric sulfate in which sulfur is in the +8 valence form:

[8] $2FeS_2 + 7.5\ O_2 + H_2O \xrightarrow{bacteria} Fe_2(SO_4)_3 + H_2SO_4$

Ferric iron is also an oxidizing agent which contributes to the dissolution of metal sulfides, for example, CuS according to:

[9] $CuS + Fe_2(SO_4)_3 \longrightarrow CuSO_4 + 2\ FeSO_4 + S^\circ$

In the presence of bacteria, ferrous iron and elemental sulfur liberated in reaction [9] will be oxidized to:

[10] $2\ FeSO_4 + H_2SO_4 + 0.5\ O_2 \xrightarrow{bacteria} Fe_2(SO_4)_3 + H_2O$

and,

[11] $S + 1.5\ O_2 + H_2O \xrightarrow{bacteria} H_2SO_4$

The sulfuric acid produced in the metabolic reactions [8 & 11] may also react with the oxide and carbonate metallic constituents of sediments to yield further metal solubilization:

[12] $MO + H_2SO_4 \longrightarrow MSO_4 + H_2O$

[13] $MCO_3 + H_2SO_4 \longrightarrow MSO_4 + H_2O + CO_2$

where M is a bivalent heavy metal. Reactions [8,10, and 11] represent the direct leaching mechanisms of bacterial action. The energy available from these oxidation reactions will be captured by the microorganisms to cover their energetic needs. Reactions [9,12, and 13] represent the indirect mode of bacterial leaching activity, where the metabolites (ferric sulfate and sulfuric acid) react with the insoluble oxide and carbonate inclusions of the sediments.

The literature of bioleaching is vast and is described in a large number of review articles (*13,15,16,18,20-23*) and symposium proceedings (*11,12,16,24-30*). The information available from these sources is relevant to the treatment of the Great Lakes sediments. The review of the above information is not the purpose of the present article. However, the authors thought to be important to include here these references for the benefit of those scientists who would like to familiarize themselves with the potential possibilities of this emerging technology.

Effects of Semiconductor Character of Metal Sulfides

It was observed by many investigators that in some cases bioleaching of particular MS is easy, and the same type of MS from a different location is very difficult. For example for chalcopyrite leaching, it was suggested that the composition $Cu(II)Fe(II)S_2$ is easy to leach, but when it is in the form $Cu(I)Fe(III)S_2$ crystallographic modification then its biooxidation is very difficult and slow. Other investigators (*31*) reported that chalcopyrite from different locations vary especially in minor and trace amounts of many elements which are present in isomorphous substitution. For example, silver, gold, platinum, lead, cobalt, nickel, manganese, tin, and zinc replace copper or iron, while arsenic, selenium and tellurium replace sulfur. In addition, chalcopyrite, as many of the MS, is a typical semiconductor material, which exhibits an energy gap of about 0.6 eV and a resistivity of about 10^{-3} ohm·m (*32*). The naturally occurring product is never perfect. It contains crystal defects (vacancies) or interstitial impurities that lead to the formation of extrinsic n-type (having an excess of negative charges) or p-type (having an excess of positive charge or holes) semiconductor. The valence energy band (lower energy band) is completely filled with electrons and these are highly bound, while the conduction band (higher energy band) is empty or only partially filled with electrons which are largely bound and free to move (*33*). Between these two bands is the energy gap, which is often called the forbidden zone of energy. The type of conductivity (n- or p- type) is determined by the energy levels of the Fermi electrons (E_f) (*34*) that can be assessed from the Hall effect. If Ef is located close to or within the conduction band, the semiconductor is designated as n-type, and in the case when the Fermi electron is located close or within the valence band, then the semiconductor is called p-type. The Fermi electron energy level represents the amount of thermodynamic work that has to be provided to the sulfide substrates (by the redox leach system of bacteria) in order to remove an electron (oxidize) the solid sulfide mineral. Therefore, the redox potential of the bacterial leach system must be higher than that represented by Ef of solid sulfide (chalcopyrite) in order for oxidation to take place (*35*).

On the basis of electron structure of the semiconductor chalcopyrite, it is likely that the n-type $CuFeS_2$ will be easier to be oxidized by the microorganisms than the p-type ore, since the electrons in the conduction zone are mobile and loosely bound. This is the reason why chalcopyrite from different localities has varying leachabilities.

INEL's Bioleaching Capabilities

In the past seven years INEL has been intensively involved in diverse bioleaching activities. The success in its activities rely on a multidisciplinary approach. Scientists with basic educational backgrounds in microbiology, biochemistry, molecular biology, genetics, chemistry, and engineering work within the disciplines of biotechnology, metallurgy and chemical engineering and collaborate to solve bioleaching problems. In the biohydrometallurgical section recently the following main topical areas have been investigated:

a) Mechanistic aspects of biocorrosion of copper with exopolymers from *Pseudomonas atlantica* indicated that copper was oxidized and a metal film was eroded as measured by FTIR/ATR coupled with XPS/AES (*36*).

b) Kinetics of biological cobaltite solubilization (*37*).

c) Identification of sulfur and iron oxidizing enzymes from *Thiobacillus ferrooxidans*.

d) Characterization of plasmids from *T. ferrooxidans* for metal specificity or heavy metal tolerance.

e) Carbon-fixation efficiency.

f) Biosorption studies (protein and exopolymer isolation, characterization, thermodynamic measurements).

Part of the above mentioned research has been supported by the U.S. Bureau of Mines and has been directed at determining the mechanisms as well as the rate and extent of biologically assisted mineral leaching and recovery from low grade ores.

The INEL laboratory has the following selected specialized equipment for conducting biotechnological research:

Atomic Absorption Spectrometer
Mobile Pilot-Scale Bioreactors
Laminar Flow Hoods
Scanning UV Spectrophotometer
GCs and HPLCs
Image Analysis System
Ion Chromatography
Ultracentrifuge
Environmental Incubator-Shakers
Electrophoretic Gel Sequencing Equipment
Fluorescent Phase Contrast Microscopes
Anaerobic Chamber
Walk-in Environmental Chamber
Bioreactors (column, airlift, RBCs)

Conclusion

The bioremediation of toxic heavy metal contents of Great Lakes sediments with iron and sulfur oxidizing microorganisms is feasible. The bio-mediated extraction processes involve the direct and indirect leaching mechanisms of bacterial action. INEL's biotechnological laboratories are well equipped with all analytical tools to cope with the complexity of bioremediation problems.

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References

1. Malcolm, S.J. and S.O. Stanley (1982). The Sediment Environment. **In:** *Sediment Microbiology*, D.B. Nedwell and C.M. Brown (eds.), Academic Press, New York, pp. 1-14.

2. Krumbein, W.C. and R.M. Garrels (1952). Origin and Classification of Chemical Sediments in Terms of pH and Oxidation-Reduction Potentials. *Journal of Geology*, **60:** 1-33.

3. Hallberg, R.O. (1980). In-situ Experimentation with Anaerobic Sediments: Some Biogeochemical Applications. **In:** *Biogeochemistry of Ancient and Modern Environments*, P.A. Trudinger, M.R. Walter, and B.J. Ralph (eds.), Australian Academy of Science, Canberra, Australia, pp. 145-155.

4. Philp, R.P., M. Calvin, S. Brown, and E. Yang (1978). Organic Geochemical Studies on Gerogen Precursors in Recently-Deposited Algal Mats and Oozes. *Chemical Geology*, **22:** 207-231.

5. Manshan, S.E. (1979). *Environmental Chemistry*, Willard Grant Press, Boston, Massachusetts, pp. 115-137.

6. Gamble, D.S. and M. Schnitzer (1973). The Chemistry of Fulvic Acid and Its Reactions with Metal Ions. **In:** *Trace Metals and Organic Interactions in Natural Waters*, P.C. Singer (ed.), Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan, pp. 265-302.

7. Peck, H.D., Jr. (1984). Physiological Diversity of the Sulfate Reducing Bacteria. **In:** *Microbial Chemoautotrophy*, W.R. Strohl and O.H. Tuovinen (eds.), Ohio State University Press, pp. 309-335.

8. Fischer, U. (1988). Sulfur in Biotechnology. **In:** *Biotechnology*, H.J. Rehm and G. Reed (eds.), VCH Verlagsgesellschaft, Weinheim, Federal Republic of Germany, volume 6B, pp. 463-496.

9. ZoBell, C.E. (1946). Studies on Redox Potential of Marine Sediments. *Bulletin of the American Association of Petroleum Geologists*, **30:** 477-513.

10. Personal communication with Paulette Altringer, (1990). U.S. Bureau of Mines, Salt Lake City Research Center, Salt Lake City, Utah.

11. Ehrlich, H.L. (1981). *Geomicrobiology*, Marcel Dekker, Inc., New York, pp. 1-393.

12. Krumbein, W.E. (1983). *Microbial Geochemistry*, Blackwell Scientific Publications, Oxford, England, pp. 1-330.

13. Ludgren, D.G. and W. Dean (1979). Biogeochemistry of Iron. **In:** *Biogeochemical Cycling of Mineral Forming Elements*, P.A. Trudinger and D.J. Swaine (eds.), Elsevier, Amsterdam, pp. 211-251.

14. Silver, M. (1978). Metabolic Mechanisms of Iron Oxidizing Thiobacilli. **In:** *Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena*, L.E. Murr, A.E. Torma, and J.A. Brierley (eds.), Academic Press, New York, pp. 3-17.

15. Torma, A.E. (1988). Leaching of Metals. **In:** *Biotechnology*, H.J. Rehm and G. Reed (eds.), VCH Verlagsgesellschaft, Weinheim, Federal Republic of Germany, pp. 367-399.

16. Karavaiko, G.I. (1985). *Microbiological Processes for the Leaching of Metals from Ores*, A.E. Torma (ed.), United Nations Environment Pro., Moscow, USSR, pp. 1-69.

17. Wichlacz, P.L. and R.F. Unz (1982). Microbiology of Coal Mine Drainage Treatment," *Proceedings 64th CIC Coal Symposium*, pp. 199-208.

18. Lundgren, D.G. and M. Silver (1980). Ore Leaching by Bacteria. *Annual Review of Microbiology*, **34:** 263-283.

19. Dugan, P.R. (1989). Microbial Conversion of Sulfur and Their Potential for Bioprocessing Fossil Fuels. **In:** *Processing of Fossil Fuels Workshop*, P.E. Bayer (ed.), U.S. Department of Energy, Washington, D.C., pp. 2-40.

20. Ralph, B.J. (1986). Geomicrobiolgy and the New Technology. *Developments in Industrial Microbiology*, **26**: 23-59.

21. Brierley, C.L. (1978). Bacterial Leaching. *CRC Critical Reviews in Microbiology*, November, pp. 207-262.

22. Smith, A.J. and D.S. Hoare (1977). Specialist Phototrophs, Lithotrophs, and Methylotrophs Unity among a Diversity of Procaryotes? *Bacteriological Reviews*, **41:** 419-448.

23. Ehrlich, H.L. (1986). What Types of Microorganisms are Effective in Bioleaching, Bioaccumulation of Metals, Ore Beneficiation and Desulfurization of Fossil Fuels. *Biotechnology and Bioengineering Symposium*, number 16, pp. 127-137.

24. Swartz, W. (ed.), (1977). *Conference Bacterial Leaching*, Verlag Chemie, Weinheim, pp. 1-270.

25. Murr, L.E., A.E. Torma, and J.A. Brierley (1978). *Metallurgical Application of Bacterial Leaching and Related Microbiological Phenomena*, Academic Press, New York, pp. 1-526.

26. Trudinger, P.A., M.R. Walter, and B.J. Ralph (1980). *Biochemistry of Ancient and Modern Environments*, Australian Academy of Science, Canberra, Australia, pp. 1-723.

27. Rossi, G. and A.E. Torma (1983). *Recent Progress in Biohydrometallurgy*, Associazione Mineraria Sarda, Iglesias, Italy, pp. 1-752.

28. Strohl, W.R. and O.H. Tuovinen (1986). *Microbial Chemoautotrophy*, Ohio State University Press, Columbus, Ohio, pp. 1-351.

29. Lawrence, R.W., R.M.R. Branion and H.G. Ebner (1986). *Fundamental and Applied Biohydrometallurgy*, Elsevier, Amsterdam, pp. 1-501.

30. Norris, P.R. and D.P. Kelly (1988). *Biohydrometallurgy*, Science and Technology Letters, New Surrey, England, pp. 1-578.

31. Habashi, F. (1978). *Chalcopyrite Its Chemistry and Metallurgy*, McGraw-Hill, New York, pp. 1-165.

32. Crundwell, F.K. (1988). The Influence of the Electronic Structure of Solid on the Dissolution and Leaching of Semiconducting Sulphide Minerals. *Hydrometallurgy*, 21: 155-180.

33. Shuey, R.T. (1975). *Semiconducting Ore Minerals*, Elsevier, Amsterdam, pp. 26-318.

34. Solymar, L. and D. Walsh (1988). *Lectures on the Electrical Properties of Materials*, Oxford University Press, Oxford, England, pp. 7-327.

35. Choi, W.K., Z.F. Wang, and A.E. Torma (1990). *Electrochemical Aspects of a Refractory Gold Ore Leaching by Thiobacillus ferrooxidans*, Reprint No. 90-159, Society for Mining, Metallurgy, and Exploration, Inc., Littleton, Colorado, pp. 1-8.

36. Gianotto, A.K., P.L. Wichlacz, J.G. Jolley, M.R. Hankins, G.G. Geesey, and R.B. Wright (1989). The Biocorrosion of Copper by Biopolymers as Examined *In Situ*, In Real Time FT-IR/ATR in Conjunction with Pre and Post XPS/AES. In: *Biotechnology in Minerals and Metal Processing*, B.J. Scheiner, F.M. Doyle, and S.K. Kawatra (eds.), Society of Mining Engineers, Littleton, Colorado, pp. 45-51.

37. U.S. Bureau of Mines (1989). Biologically Assisted Minerals Processing. In: *Strategic and Critical Materials Program Annual Report - 1989*, Idaho National Engineering Laboratory, Department of Energy, pp. 1.1-1.12.

6.6 Linking Biological and Hydrogeochemical Mechanisms of Sediment Leaching

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Introduction

Leaching and fixation of inorganic materials in fluvial and lacustrine sediments result from hydrogeochemical and biochemical (microbial) processes. The interdependence of such mechanisms is recognized as critical. Cross-disciplinary research is difficult because of differences in vocabulary, conceptual models, and methods of experimental design. If interdisciplinary teams of scientists are to interpret the mechanisms operating in a given system, experiments must be designed so that the parameters required by scientists from diverse fields, such as microbiology, hydrology, and hydrogeochemistry, are measured. Ideally, teams would then interpret findings from new, hybrid perspectives as well as from their discipline-specific perspectives.

In order to investigate methods of bioremediation of contaminated fluvial and lacustrine sediments, it is useful for scientists to have at their disposal methods to rapidly simulate the results of a hypothetical remediation procedure. A valuable class of analytical and predictive tools is computer models. Calibration and sufficient verification of computer models makes it possible to predict qualitatively contaminant fate at new sites on the basis of knowledge gained at sites that have already been studied.

Computer models exist that use data on sediment and pore water chemistry to predict mineral solubilities, that is, to determine whether inorganic materials are fixated *in situ* in a solid matrix in an innocuous form. Other computer models consider data on biological composition. Some computer models use the hydraulic parameters of aquifers to predict spatial and temporal distributions of inorganic contaminants in ground water, while others predict contaminant transport in open estuaries. Generally, those computer models appropriate to address the interdisciplinary perspectives described above would involve consideration of the combined kinetic and equilibrium effects of chemical dissolution, biochemical dissolution, chemical fixation, biochemical fixation, and the dispersion characteristics of moving ground and surface water. No single "utopian" computer model exists that represents all these processes, nor is there all the underlying data required to run such a model if it did exist. The development of a completely linked model would require a large, multidisciplinary team, a lengthy time frame, and a massive budget. At this time, it is only reasonable to use existing models separately, with experienced scientists acting as the links, or interface, among the models.

Background

Staff from the U.S. Bureau of Mines' Spokane Research Center studied sulfide oxidation, metal leaching, and metal transport in the unsaturated and saturated zones of fine-grained tailings and a downgradient aquifer. Water quality data were interpreted using an equilibrium geochemistry computer model, verifying the hypothesis that certain minerals (inorganic chemical compounds) would precipitate, others would dissolve, and yet others would tend to exert solubility controls on concentrations of various constituents within certain pH ranges. An additional finding was that an organic layer at the base of the waste impoundment may be causing the attenuation of metals transported from the tailings.

Key questions remaining in the interpretation of sulfide oxidation at this site include: What microbial species are present in the tailings? What biochemical effects would these populations have upon the system? Are different microbes present in the organic layer than are present elsewhere? Are the microbes that thrive in the variably saturated (air and water) zone aerobic or anaerobic? Does microbial activity vary seasonally? How do microbes catalyze (increase the kinetics, or speed of) oxidation reactions occurring within the tailings? Are any microbes acting to fix metals further downgradient where metal concentrations diminish? For example, the oxidation of ferrous to ferric iron can be catalyzed by the bacterium *Thiobacillus ferrooxidans*. This catalysis may increase the reaction rate by as much as five to six orders of magnitude. Hydrogeochemists know of the implications of this reaction but generally do not quantify it. Other possible microbial mechanisms are rarely considered in interpretations of the chemistry of a site. Cooperative work with microbiologists at this site will be directed to addressing more such processes.

Part I: Field and Laboratory Data Requirements

In order to determine the interdependence of hydrogeochemical, hydrological, and microbiological mechanisms in the bioremediation of contaminated fluvial and lacustrine sediments, data should be collected to address the following questions:

1. What types and amounts of inorganic species can be leached from a matrix of specific composition by pore water of specific composition and known chemical parameters? At what rates?
2. What types and amounts can be leached by biota catalysis? At what rates?
3. What types and amounts can be chemically precipitated? At what rates?
4. What biomediated fixation processes are occurring? At what rates and in what quantities?
5. How are dispersion, dilution, and sorption properties quantified in an aquifer or estuary?
6. What are the hydraulic properties of an aquifer or estuary?

Questions 1 and 4 are complicated by the necessity of using kinetic (reaction rate) as well as equilibrium (maximum reaction extent and direction) considerations.

The data required to characterize these interdependent processes are as follows:

Hydrogeochemistry

The field data required for input into most hydrogeochemistry models are relatively standard and straightforward. Measurements are made for pH, Eh (redox potential), temperature, and electrical conductivity. Additional measurements are usually made for alkalinity and reactive dissolved gases such as O_2, NH_3, SO_2, and CO_2, which are dependent upon site-specific requirements. Most of these field measurements (with the exception of temperature, alkalinity, and conductivity) are made with potentiometric electrodes. Another type of potentiometric electrode, the ion-specific electrode, can measure a limited variety of mono- and divalent ions, but usually these element concentrations are determined in the laboratory. Potentiometric methods are usually only reliable and linear within specific ranges and are subject to numerous interference problems, particularly in solutions with significant levels of dissolved solids. Consequently, in certain instances, other methods, such as titration, must be used, even in the field. Ion speciation analysis is often also performed in the field if the element in question changes oxidation states rapidly. Many models utilize an Fe(II)/Fe(III) ratio, and Fe(II) can be determined readily in the field by colorimetry.

Water samples are analyzed in the laboratory for dissolved elemental or ionic constituents with instruments. The available methods and their variants are innumerable, but the majority of analyses of inorganic materials are performed using spectral emission or absorption. There is no "ideal analytical procedure" for any given substance; the procedure developed will be controlled by site-specific conditions. Spectral interferences will vary from site to site, as will concentrations and other factors. All these factors must be evaluated when determining what analytical procedure to use. The analyses menu will also be site specific; it must be determined by a general analytical scan, a mineralogic analyses, and requirements of the biota. The latter illustrates the interrelationship of considerations of the biosphere and the hydrosphere. A substance which is of no thermodynamic or kinetic consequence may be of extreme biochemical importance as an energy source or as a substrate or biocide, and its concentration must be determined.

Mineralogic analyses (solid and dissolved phases) must also be performed. In order to simulate a leaching from the solid phase, the solid phase must be identified and quantified. Again there is no "ideal method" for a given substance, and site-specific influences will determine the suite of techniques selected. Reflective or transmissive optical methods can be used to identify many inorganic particles, but identifying many amorphous substances will require more expensive and sophisticated techniques, such as x-ray scanning or ion-microprobe/SEM analysis. Identification of organometallic complexes can be even more perplexing, and infrared scanning, nuclear magnetic resonance, chromatography, or mass spectrometric methods, among others, are often necessary. In certain situations, such as with solid-solution minerals, the composition of the mineral as well as its identity must be determined.

Biochemistry

The data requirements for characterizing microbiological processes include the rates at which microorganisms catalyze reactions. These rates are a function of temperature, initial population density, and the availability and concentration of energy sources and substrates such as oxygen, nitrogen, sulfur, carbon, and phosphorus compounds. The concentrations and identities of the mineral or substance to be catalyzed must be known, and the levels of any biocides (or rate inhibitors) should be determined. Unfortunately, the identities of inhibitors for many microbes are not known. The reaction rate will also be a function of mineral surface area; therefore, a particle size distribution analysis must be performed. This information will also be required to determine purely chemical reactions.

Perhaps most important is information about the microbes themselves. Important classes of microorganisms include those free-floating in the aqueous phase and those attached to the surfaces of the solid phases. The identities, sizes, and population densities of organisms associated with all phases must be determined. This means that for the solid phase, each organism for each mineral of interest must be identified, because the type and density of microbes vary with mineralogy. An added complication is that the activities of the microbe population must also be determined. A microbe may be present, but it will not participate in a reaction unless it is activated. Pulsing cycles are likely to occur if a microbial population grows exponentially, exhausts an energy source, and dies off. When new nutrients are supplied by a water-transport process, the population may be reestablished. For microbes that incorporate liberated inorganic material into their structure, the attrition rate must be known. If these organisms die, a contaminant could be much more difficult to extract from the system. Attrition could be the end of the normal life cycle or be caused by biocides; also, the microorganism could be consumed by a more complex life-form that accumulates the contaminant.

Hydrology

The hydrologic setting of a bioremediation effort may be in a groundwater aquifer or an open estuary. The hydrogeologic parameters necessary to perform flux calculations in groundwater are relatively well understood, and the calculations are reasonably well developed and proven. Collection of adequate data to support such models, however, can be quite expensive. The geometry and internal structure of the sediment mass of interest must be established. Flow direction and rate are, to a great extent, determined by the stratigraphy of the sediment and are determined by surface and bathymetric mapping, geophysical surveying, and well installation (for potentiometric head measurements). Hydraulic head distribution within each individual stratigraphic unit in the sediment will indicate the natural flow direction and flux of water under static conditions. Vertical and horizontal coefficients of hydraulic conductivity for each stratigraphic unit can be determined through aquifer tests in the field or by permeameter testing of properly prepared undisturbed samples. Horizontal hydraulic conductivity tends to be much greater than vertical hydraulic conductivity in sedimentary units because of the tendency of mineral grains to interlock in a horizontal direction during sedimentation. All porous-media models, analytic or numeric, use Darcy's Law, $Q = -KA\,(dh/dl)$, as the primary governing equation.

Contaminant flux in lakes and estuaries is dominated by fluid mechanics. Mixing and dispersion are fueled by temperature or salinity density differences, deltaic processes, and tidal movement.

Part II: Computer Model Requirements

There are two types of computer models: analytic and numeric. Analytic models are essentially exact calculations and are not easily adapted to spatial and temporal variability. Numeric models are based on successive iterations of controlling formulas and adapt well to real-world conditions where time and space are varied during simulations. A detailed discussion of model construction is beyond the scope of this presentation, and the reader is referred to the bibliography.

A number of models exist that contain various subsets of the components described above. MINTEQ (U.S. Environmental Protection Agency) and WATEQ and PHREEQE (U.S. Geological Survey) compare water composition and chemistry to thermodynamic equilibrium data bases to forecast the tendency of certain minerals to precipitate or dissolve. BALANCE (U.S. Geological Survey) makes mass balance calculations of changes in water chemistry from single or dual sources.

Additional models are EQ6 (Lawrence Livermore National Laboratories), which uses a limited reaction rate data base to predict mineral precipitation; CHEMTRN and TRANQL, which link equilibrium chemistry with mass balance calculations; and FASTCHEM (Electric Power Research Institute), which links equilibrium chemistry and mass balance calculations with advective transport, sorption, and dispersion. FOWL (Electric Power Research Institute), predicts leachant concentrations from fly ash impoundments, but it is based on an empirical, not a thermodynamic, data base. RATAP (CANMET) utilizes very limited biochemical, equilibrium, and kinetics data bases to predict the dissolution rates of pyrite, pyrrhotite, chalcopyrite, and sphalerite in mine tailings ponds. Obviously, there is a dearth of models that incorporate biochemistry. A wide variety of ground water flow models is available; one of the most commonly used is MODFLOW3D (U.S. Geological Survey), but more sophisticated finite-element models are now available. Most of the models mentioned need fine tuning and field verification under widely variable conditions, but there is a serious shortage of trained personnel to do so.

The modular numeric model is the type that might be developed to link hydrogeochemical, biochemical, and hydrologic models as modules into one large, complex program. In each iteration, most variables would be held constant while solving for others, but eventually, every change of every variable in each module would affect many other variable values. Such changes might be small or large, inversely proportional or directly proportional, linear or exponential. Such a model would be necessarily constantly testing for limiting factors, such as depletion of a substrate or the level of a biocidal constituent. The model would have to search data bases constantly to fill variables, such as thermodynamic constants, and it would have to monitor surprising items, such as the decrease in aquifer hydraulic conductivity induced by the filling of voids in the event of a burgeoning microbe population. No such model exists, but several groups (e.g., Battelle National Laboratories and the U.S. Geological Survey) are developing smaller programs that could become modules in such a model. A fully linked model that begins with a steady state, combines the compositional and biologic data of aquifer material, pore water, and injection fluid with hydrogeologic data to predict the growth and leaching characteristics of the microbes and the leaching fluid and the composition of the fluid at any point in time and space may be beyond the time, budget, and personnel availability of any industry. There is a severe lack of the most basic research; the data bases necessary to supply equations with constants are usually incomplete, inaccurate, unproven, or nonexistent. Even if such a model could be developed, its output would be only as good as the site-specific input data, and user costs of collecting this data may be prohibitive. Users with partial input data sets might be tempted to use a model with default values or use published data that are not necessarily transferable. It is then impossible to determine the defensibility of the output. Model users frequently fall into this trap.

Beyond field verification and improvements to existing models of all varieties, the greatest need for future research is in the area of developing new chemical and biochemical data bases and improving existing ones. Thermodynamic equilibrium data bases have been reasonably well developed, but they need to be supplemented with additional minerals, and the data for many minerals is suspect. Kinetic data bases for oxidation/reduction and precipitation reactions are extremely limited, and the kinetics are poorly understood. Biochemical data bases are essentially nonexistent, and these will probably be the most difficult and expensive to develop. The inventory of microorganisms involved in the dissolution and fixation of minerals and dissolved contaminants is extremely incomplete, and little is known of the nature of the fixation mechanisms. Nor do we understand completely population growth rates in the presence of various substrates, energy sources, and biocides. Obviously, there are many unanswered questions. What will happen to theoretical reaction rates when a myriad of microorganic and chemical mechanisms compete for the same reaction? How should this phenomenon be quantified? At what population density do stearic effects induce nonlinearity in biochemical reactions, and how is this modeled? What organisms incorporate inorganic contaminants in their cell structure and in what amounts?

These shortcomings must be overcome before any serious efforts at developing a fully integrated equilibrium/kinetic-biochemical-hydrogeologic ground water model can begin.

Conclusions

Adequate information is not available to link biochemical and hydrochemical mechanisms realistically in a single model. Future research must be concentrated toward developing new biochemical data bases and better equilibrium/ kinetic data bases, determining how chemical and biochemical mechanisms interact, and determining whether it is feasible to incorporate all components into a dynamic transport model. The resulting program would be modular, require a very powerful computer, and be very expensive and time consuming to develop. The only defensible short-term approach is to interpret the results of several existing biochemical and hydrogeochemical models jointly, with experienced scientists acting as the link, or interface, among the models.

References

1. Association of Ground Water Scientists and Engineers (1987). *Proceedings, Solving Ground Water Problems with Models, Conference and Exposition: Vol. 1,* Denver, CO, February 10-12, 1987.

2. Ball, J. W., and D. K. Nordstrom (1987). *WATEQ4F--A Personal Computer Fortran Translation of the Geochemical Model WATEQ2 with Revised Data Base.* U.S. Geological Survey Open-File Report 87-50.

3. Canada Centre For Mineral And Energy Technologies (1988). *Adaptation of the Reactive Acid Tailings Assessment Program (RATAP) to Base Metal Tailings.* Contract #15SQ-2344-7-9208.

4. Criscenti, L. J., M. L. Kemner, R. L. Erikson, C. J. Hostetler, J. R. Morrey, and J. S. Fruchter (1989). *The FASTCHEM Workstation for Pre- and Postprocessing Functions.* Battelle Pacific Northwest Laboratories for the Electric Power Research Institute. EPRI EA-5870.

5. Davis, J. A., and K. F. Hayes (eds.) (1986). *Geochemical Processes at Mineral Surfaces.* 190th Meeting of the American Chemical Society. Chicago, IL, September 8-13, 1985. ACS Symposium Series 323. American Chemical Society, Washington, D.C.

6. Hem, J. D. (1985). *Study and Interpretation of the Chemical Characteristics of Natural Waters.* U.S. Geological Survey Water-Supply Paper 2254.

7. Hostetler, C. J., R. L. Erikson, J. S. Fruchter, and C. T. Kincaid (1989). *FASTCHEM Package: Vol. 1-5.* Battelle Pacific Northwest Laboratories for the Electric Power Research Institute. EPRI EA-5870.

8. Krupka, K. A., R. L. Erikson, S. V. Mattigod, J. A. Schramke, and C. E. Cowan (1988). *Thermochemical Database Used by the FASTCHEM Package.* Battelle Pacific Northwest Laboratories for the Electric Power Research Institute. EPRI EA-5870.

9. McDonald, M. C., and A. W. Harbaugh (1984). *A Modular Three-Dimensional Finite Difference Ground-Water Flow Model.* U.S. Geological Survey.

10. National Research Council (1990). *Ground Water Models: Scientific and Regulatory Applications.* National Academy Press, Washington, D.C.

11. Parkhurst, D. L., L. N. Plummer, and D. C. Thorstenson (1982). *Balance - A Computer Program for Calculating Mass Transfer for Geochemical Reactions in Ground Water.* U.S. Geological Survey Water-Resources Investigations 82-14.

12. Patterson, J. W., and R. Passino (eds.), (1987). *Metals Speciation Separation and Recovery. Proceedings, International Symposium on Metals Speciation, Separation, and Recovery.* Chicago, IL, July 27-Aug. 1, 1986. Lewis Publishers, Inc.

13. Schwab, A. P., R. L. Schmidt, D. C. Girvin, and J. E. Rogers (1984). *Chemical Attenuation Rates, Coefficients, and Constants in Leacheate Migration. Vol. 1: A Critical Review.* Battelle Pacific Northwest Laboratories for the Electric Power Research Institute. EPRI EA-5870.

14. Singer, P. C., and W. Stumm (1970). Acid Mine Drainage: The Rate-Determining Step. *Science,* **167:** 1121-1123.

15. Sumners, K. V., S. A. Gherini, M. M. Lang, M. J. Ungs, and K. J. Wilkinson (1989). *MYGRT Code Version 2.0: An IBM Code for Simulating Migration of Organic and Inorganic Chemicals in Groundwater.* Tetra Tech, Inc. for the Electric Power Research Institute. EPRI EN-6531.

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Appendix I - PROGRAM

Biological Remediation of Contaminated Sediments with Special Emphasis on the Great Lakes

July 17 - 19, 1990
at the Inn on Maritime Bay
Manitowoc, Wisconsin

Tuesday, July 17

7:30 - 8:30 A.M. Registration; Coffee and Doughnuts

8:30 - 8:45 A.M. Introductory Remarks

8:45 - 12:00 Noon **SESSION I.** Overview of 5 Primary Areas of Concern
Session Moderator: Dave Cowgill

8:45 Indiana Harbor AOC, Robert Bunner

9:15 Field Brook-Ashtabula River Superfund Site and AOC, Pete Sanders

9:45 Buffalo River AOC, John McMahon

10:15 Break

10:35 Sheboygan River Superfund Site and AOC, Bonnie Eleder

11:05 Saginaw River AOC, Greg Goudy

11:35 Roundtable Discussion.

12:00 Noon **GROUP LUNCHEON**, at the Inn

1:30 - 5:30 P.M. **SESSION II.** Laboratory and Field Studies: Biological Degradation of PCB's
Session Moderator: John E. Rogers

1:30 Dechlorination of Arochlors by Anaerobic Bacteria in Sediments, John F. Quensen III

2:15 Aerobic Biodegradation of PCB's, Ronald Unterman

3:00 Break

3:30 Anaerobic Biotransformation of PCB's in Sediments, G-Yull Rhee

4:15 Remediation Pilot Study in the Sheboygan River Wisconsin, USA, Dawn Foster

5:00 Roundtable Discussion

Wednesday, July 18

8:00 - 8:30 A.M. Coffee and Doughnuts

8:30 - 12:00 Noon **SESSION II (continued).** Laboratory and Field Studies: Biological Degradation of PCB's and PAH's

8:30 Sequential Anaerobic - Aerobic Biodegradation of PCB's, Daniel Abramowicz

9:15 PCB Dechlorination of the Sheboygan River, William Sonzogni

10:00 Break

10:30 PAH Contamination of Hamilton Harbor, Tom Murphy

11:15 Roundtable Discussion

12:00 Noon LUNCH, on your own

1:30 - 5:30 P.M. **SESSION III.** Biological Treatment of Metal Species
Session Moderator: Paulette Altringer

1:30 Bacterial Leaching of Metals from Various Matrices found in Sediments, Removing Inorganics from Sediment-Associated Waters Using Bioaccumulation and/or BIOFIX Beads, Paulette Altringer

2:15 Use of Wetlands and Anaerobic Bacteria to Remove Metals From Acid Mine Drainage, and Bactericides to Deactivate Leaching Reactions, Hank Edenborn

2:55 Bioleaching of Manganese, Platinum, and Gold Ores, Betty Baglin

3:15 Break

3:45 Mechanisms of Bacterial Metals Removal From Solids, Arpad Torma and Pete Pryfogal

4:25 Linking Biological and Hydrogeochemical Mechanisms of Sediment Leaching; I. Field and Laboratory Data Requirements, and II. Computer Model Requirements, Bob Lambeth

5:05 Roundtable Discussion

6:30 P.M. **GROUP DINNER** at the Inn
Address: EPA's Research Program on Biological Remediation of Alaskan Beaches following the Valdez Oil Spill, John E. Rogers

Thursday, July 19

8:00 - 8:30 A.M.	Coffee and Doughnuts
8:30 - 12:00 Noon	**SESSION IV**. Laboratory and Field Studies: Biological Degradation of PAH Compounds Session Moderator: Chad T. Jafvert
8:30	The Use of *Mycobacterium species* in the Remediation of PAH Waste, Carl Cerniglia
9:15	State-of-the-Art Sediment Remediation in The Netherlands. Biological Remediation of PAH compounds, H. J. van Veen
10:00	Break
10:20	Recent Studies on the Microbial Degradation of PAH's and Their Relevance to Bioremediation, Dr. James Mueller (presented by John Rogers)
11:05	Fungal Degradation of PAH's, John Glaser
11:50	Roundtable Discussion
12:20 P.M.	ADJOURN

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Appendix II - List of Attendees

Workshop on
Biological Remediation of Contaminated Sediments
with Special Emphasis on
the Great Lakes

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